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Microbiological risk assessment in feedingstuffs for food-producing animals[1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-045)

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PANEL MEMBERS

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SUMMARY

Following a request from the Health and Consumer Protection, Directorate General, European Commission, the Panel on Biological Hazards was asked to deliver a scientific opinion on Microbiological Risk Assessment in feedingstuffs for food-producing animals for both public health and animal health. In accordance with the terms of reference, this report does not consider hazards such as BSE/TSE agents, parasites and virus, or contamination by fungi and mycotoxins.

The Panel on Biological Hazards identified *Salmonella* spp. as the major hazard for microbial contamination of animal feed. *Listeria monocytogenes*, *Escherichia coli* O157: H7 and *Clostridium sp*. are other hazards for which feed is regarded a far less important source. In addition, antimicrobial resistant bacteria, or antimicrobial resistance genes can be transmitted *via* feed.

Forage, industrial compound feed, home-grown cereals and purchased straight feedingstuff are the four major groups of feeds for EU livestock. The report focuses on industrial compound feed as the feed group with the highest risk for becoming contaminated by *Salmonella* spp. Oil seed meal and animal derived protein are the major risk feed materials for introducing *Salmonella* contamination to feed mills and industrial compound feed. Data of *Salmonella* contamination in forage is scarce, and in most studies non-processed cereals are reported to have a low prevalence of *Salmonella* spp, while available data demonstrates that non-processed soybeans are often contaminated with *Salmonella*. As there is limited information on the occurrence of *Salmonella* associated with home-mixing of feeds, the Panel on Biological Hazards recommended that more information should be gathered on the proportion of feed which is home-mixed for the various livestock species in EU MS, and to identify the sources of feed materials and procedures used by home-mixers, which may contribute to contamination with *Salmonella*. Overall, comparable data on *Salmonella* in feed production at the EU level

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should be obtained, preferably by means of a base line survey (including information about prevalence in feed materials, compound feed and details of the production processes). These data could then be used to inform decisions to improve control of *Salmonella* in feed production.

Animals can become infected when fed with *Salmonella*-contaminated feed. This may cause occasionally clinical disease in some animals, but the major outcome is asymptomatic carriage. In addition, animals may also become infected from other *Salmonella*-infected animals, directly or via a contaminated environment for which the original source could have been contaminated feed. Transmission of *Salmonella* from animal feed to animals consuming the feed, and to food products derived from the animals has been shown. The relative importance of different sources of *Salmonella* infections in animals varies. In regions with low prevalence status, where endemic infection is well controlled or absent, *Salmonella* contaminated feed is the major source for introducing *Salmonella* into the animal food production. In other regions with high prevalence, although it is difficult to quantify, the relative importance of feed as compared to other sources of *Salmonella* may be lower. In all situations, there is a possibility of introducing *Salmonella* in animal production via feed, which would compromise the results of other control measures. Although the most common *Salmonella* serotypes occurring in humans are seldom found in animal feedstuffs in most countries, some serotypes found in feed are also found in humans.

The feed production industry has a relevant role in the food chain. In order to ensure production of safe feed, EC Regulation 183/2005 indicates that "Feed business operators shall put in place, implement and maintain permanent written procedures based on the HACCP principles". The European Feed Manufacturers Guide and the Feed Ingredients Standard were published as a guide to good practices.

There are safety benefits from the application of HACCP principles, GHP and GMP approaches in animal feed production. The Panel on Biological Hazards recommended that effective implementation of HACCP principles, and GMP/GHP procedures along the feed chain should be ensured. This requires proper control of recontamination, as well as determination of the effective heat treatments at the individual plants. The importance of starting the control already at the crushing and the rendering plants is emphasised.

Moist heat can effectively decontaminate feed materials, as well as compound feed as long as sufficiently high temperatures and treatment times are used. Where GHP/GMP are in place, the risk of recontamination is minimised. Comparative studies suggest that heat treatment processes used to successfully control *Salmonella* contamination will also be effective for other non spore-forming food-borne pathogens. Although heat treatment is generally recognised as the most effective decontamination method, in some circumstances (e.g. pelleted feed for layers) this may not be appropriate. In such cases, chemical treatment of feed may offer an alternative means of protection. Treatment of feed ingredients or compound feed with blends of organic acids, or with formaldehyde products at suitable concentrations, can be effective in reducing contamination by *Salmonella* spp. and other organisms. Furthermore, chemical treatment has a residual protective effect in feed, which helps reduce recontamination and also helps reduce contamination of milling and feeding equipment and the general environment. The Panel on Biological Hazards recommended that more research is led on the relative efficiency of chemical feed decontaminants and their effect on subsequent *Salmonella* status of animals fed on treated rations. Furthermore, a standard test model is required for chemicals used for decontamination of feed.

The aim is for the feed manufacturer to continuously reduce the occurrence of *Salmonella* in feed for all food-production animals. Establishment of microbiological criteria for *Salmonella* contamination along the feed chain is appropriate and suggested as one of several tools. A feed

safety criteria based only on testing of the end product would not be an effective way to ensure absence of *Salmonella* contamination. Therefore, establishment of one or more process hygiene criteria at critical stages of the feed production chain, including at the end product stage, is more efficient. The Panel on Biological Hazards recommended that common EU process hygiene criteria should be established on crushing plants, rendering plants and feed mills as an integrated part of specific HACCP-based control programmes to maximise the control of *Salmonella* contamination for all food-production animal species. In addition, the ISO6579:2002 Annex D based method, which has been adopted as the EU standard method for monitoring zoonotic *Salmonella* spp., should urgently be validated for use in feed. Any alternative method should be equally validated for use in feed.

Key words: Feed, *Salmonella*, control options, microbiological criteria

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BACKGROUND AS PROVIDED BY HEALTH AND CONSUMER PROTECTION, DIRECTORATE GENERAL, EUROPEAN COMMISSION

Regulation (EC) No 183/2005 on feed hygiene lays down obligations for feed business operators and applies since 1 January 2006. In accordance with Article 5(3) of this regulation, specific microbiological criteria and targets shall be adopted. Certain microbiological criteria for feedingstuffs are set in the national legislation of some Member States. However, these criteria are not harmonized in the European Union for the majority of feedingstuffs, the only exceptions being maximum contents and guidance values for mycotoxins and requirements for animal by-products that can be used as feed materials and petfood, which are laid down in Regulation (EC) No 1774/2002 concerning animal by-products not intended for human consumption.

Therefore the European Commission is considering the establishment of specific microbiological criteria and targets for feedingstuffs for food-producing animals in Community legislation on feed hygiene. These criteria would have to take into account the impact on public health and on animal health. In support of this, Community legislation has to be underpinned by scientific advice. To this end, the European Food Safety Authority (EFSA) should be consulted.

Feedingstuffs comprise several types of substances and products used for feeding of animals: feed materials (for which a non-exclusive list is laid down in Council Directive 96/25/EC of 29 April 1996), feed additives, pre-mixtures and compound feedingstuffs. Compound feedingstuffs are not the only source of feed in the Community. Cereals provide an important contribution to the rations. Grazing animals feed on forage and roughage. The presence of certain microorganisms such as *Salmonella* in compound feedingstuffs can often be traced back to feed materials, although cross-contamination in storage, transport and processing is not excluded.

In accordance with Regulation (EC) No 2160/2003, targets for reduction of prevalence are being set for *Salmonella* serotypes with public health relevance in different animal populations (breeding hens, laying hens, broilers, turkeys, slaughter pigs and breeding pigs). Also Member States' competent authorities have identified *Salmonella* as a priority for setting microbiological criteria and targets for feed. Therefore the impact of the contamination of feedingstuffs on the prevalence in animal populations should be evaluated.

Furthermore, according to the opinion of the BIOHAZ Panel on "Risk assessment and mitigation options of *Salmonella* in pig production" from 16 March 2006, "the control of *Salmonella* contamination of feed is essential […]". It is also stated that "considerable efforts are required to limit exposure of *Salmonella* from feed to an absolute minimum". In addition, the role of "[…] *Salmonella* contaminated feed as a continuous risk for new introduction to herds in all Member States should be considered for further action", as indicated in the "Review of the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004", adopted by the BIOHAZ Panel on 7 September 2006 and by the AHAW Panel on 8 September 2006.

In preparing the opinion, exposure of animals to all types of feedingstuffs for food-producing animals should be considered: feed materials of plant and of animal origin, feed additives, pre-mixtures and compound feedingstuffs for different species and categories of animals.

Although all food-producing animals should be considered, the risks could be addressed separately for food-producing animal populations for which a control programme is mandatory (broilers, laying hens, breeding hens, turkeys, fattening pigs, breeding pigs) and for other farmed animals (bovines, ovines, fish, etc).

TERMS OF REFERENCE AS PROVIDED BY HEALTH AND CONSUMER PROTECTION, DIRECTORATE GENERAL, EUROPEAN COMMISSION

The European Food Safety Authority is asked to provide an opinion on microbiological risk assessment in feedingstuffs for food-producing animals for both public health and animal health. The opinion should address in particular the following:

Microbiological hazards

- Identify microbiological hazards in feedingstuffs that can pose a risk to animal health and public health, especially *Salmonella,* but also other bacteria that are pathogenic for humans and/or animals.
- To assess, and if possible to quantify, to what extent feedingstuffs contaminated with *Salmonella*[2] can contribute to:
  - the prevalence of *Salmonella* in animals and its animal health implications,
  - contamination of food produced from those animals,
  - the prevalence of *Salmonella* cases in humans.

Quantification of the effect of control options

- To assess, and if possible, quantify the effect of the most important control options, in particular:
  - good hygiene and manufacturing practice and principles based on HACCP applied to each stage of feed production chain;
  - processing conditions aimed at reducing the microbial contamination of feedingstuffs, avoiding their recontamination after processing and preventing the multiplication of microorganisms (e.g. pelleting, heat treatment, treatment with acids).
- Identify the areas where it would be appropriate to set microbiological criteria and/or targets for feedingstuffs to ensure a high level of public health and animal health protection, as well as the elements to be taken into account (such as sampling plans, analytical methods, etc.).

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[2] Although all *Salmonella* serotypes should be considered, particular attention could be given to those with higher animal health and public health significance, in particular those referred to in Regulation (EC) No 2160/2003 (*S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar).

ASSESSMENT

1. Introduction

The feeding of animals reared for food producing is very diversified, and sometimes very complex. This is due mainly to the diversity of food-producing animal species. Moreover, considerable differences are found in the production systems, with a very large range between "natural" feeding and feeding with compound feedingstuffs.

It is also important to notice that the use of by-products has a very important function for the feeding of animals; as an example, oilseed meals, which are by-products of processing seeds for oil for human consumption, are used as protein sources. Actually, in EU, oilseed meals and cakes are the second most important group of ingredients in animal feed, which are mainly used as primary source of protein (http:www.fediol.be/2/main5.php).

The constant increase on animal production at the EU level has been followed by an increase of the amount of feedingstuffs produced and given to animals. The feeding of animals can be carried out in different ways. Nevertheless, the main source is compound feedingstuffs. This type of feed comprises several types of substances and products such as: feed materials as described in the EU Regulation 96/25/EEC (http://ec.europa.eu/food/food/animalnutrition/labelling/marktlab01_en.pdf), feed additives and premixtures (see glossary).

Feeding is considered as a fundamental factor, not only for the health and welfare of animals, but also for the human nutritional intake, due to the consumption of food produced by these animals. The feeding of animals should not have a negative effect on animal and public health, but in some circumstances animal feeding have been associated with some food safety issues (e.g. BSE and salmonellosis).

The scope of this report is to assess the risk, for both animal and public health, due to the presence of pathogenic bacteria, especially *Salmonella* spp. in different feedingstuffs, and to evaluate the most important options to control the risk, including the setting of microbiological criteria or targets during the feed production chain. In accordance with the terms of reference, this opinion does not contemplate hazards such as BSE/TSE, presence of agents such as parasites and viruses, and contamination by fungi and mycotoxins. Furthermore, due to the lack of scientific information, it was not possible to quantify the contribution of feedingstuffs contaminated by *Salmonella* spp. to the prevalence of these bacteria in animals, contamination of food, and to the prevalence of *Salmonella* cases in humans. In the same way, the BIOHAZ Panel therefore proposes a qualitative assessment on different options used to control *Salmonella* spp. contamination in feed.

2. Feed production and structure (EU, imports, farm-feeding systems)

2.1. Number of food producing animals and herd size in the EU

The number of the animals in the production categories (poultry, pigs, bovine and ovine species as well as fish) and the organisation (herd and flock size, individual family farms or cooperatives and big companies, animal trade etc.) impact on the magnitude of the need for feed as well as on the mode of production and import of raw materials for the final feedstuffs that are used.

The following description of the number of animals by production categories (animal species) in the EU-27 is based on the latest internet information (the "Eurostat 2007" available at http://epp.eurostat.ec.europa.eu).

2.1.1. Cattle (dairy cows and beef cattle)

In the EU-27, 90 million head of cattle, 25 million of them are dairy cows, are kept in the end of 2007. The "old" EU-15 has 76 million head of cattle with 18 million dairy cows, the new EU member states that joined the EU in 2005 added 10 million, and the two new member states that joined the EU in 2007 added 3 million head of cattle. It is obvious that in the "old" member states (EU-15), the proportion of beef cattle (75%) is much higher than in the EU-10 that joined the EU in 2005 (50%) and the lowest in the two "newest" member states (30%).

The average herd size is varying between the member states from 2.2 head of cattle per herd in Rumania to 190.7 in Cyprus. More countries with a very small average herd size are Bulgaria (3.6), Latvia (6.8) and Poland (7.0). The countries with the biggest average cattle herd sizes are: Cyprus (190.7), Luxembourg (118.7), The Netherlands (101.8), Czech Republic (93.5), Denmark (93.1), Belgium (87.5), France (80.2, and Germany (71.1). The biggest national populations of dairy cows have Germany (4.3 million), France (3.9 million), Poland (2.6 million), United Kingdom (2.1 million), Italy (1.8 million), The Netherlands (1.4 million), Ireland (1.1 million), and Spain (1.0 million).

To approach a rough estimate of the degree of the national cattle productions being dependent on compound feed with a higher proportion of imports, an understanding of data on the national numbers of cattle per 100 ha arable land in the individual EU-27 member states is important: this number varies from 16.6 (Hungary) to 194.7 (Belgium). Further countries with a very low stocking density per 100 ha arable land, are: Greece (18.0), Rumania (19.9), Latvia (21.7), Bulgaria (22.3), Spain (23.6), and Slovakia (27.4). Further countries with very high numbers of cattle per 100 ha arable land, are: The Netherlands (194.0), Malta (193.6), Ireland (162.8), and Luxembourg (143.5).

2.1.2. Pigs (breeding and slaughter pigs)

In the EU-27, 154,507,700 pigs in total are kept at the end of 2007. This number represents the number of pigs that are there at one point in time. The number of finishing pigs has to be multiplied by 2.5 to represent the number of slaughter pigs produced per year, which is not necessarily the number of pigs slaughtered per member state - some countries "export" weaner pigs (The Netherlands, Denmark, Belgium, and Spain), some countries "import" weaner pigs (Germany, France, Italy, Poland). Germany is the country with the biggest national pig population (26.9 million) followed by Spain (22.8 million). Also "big" pork producing countries are Poland (17.7 million), France (14.8 million), Denmark (13.5 million), and The Netherlands (11.3 million). However, whereas the 13.5 million pigs in Denmark are produced by 8,890 pig producers and the 11.3 million pigs in The Netherlands by 9,690 pig producers, the 17.7 million pigs in Poland are produced by 701,660 farmers. The range of the herd sizes per in the EU-27 member states is illustrated by the list: in the following countries is the average number of pigs per holding very low: Rumania (2.8), Bulgaria (4.9), Lithuania (7.9), Latvia (11.0), Slovenia (14.9), Hungary (12.2), Portugal (22.2), Greece (22.8), Slovakia (24.1), and Poland (25.3). In contrast to this, the average number of pigs per holding is very high in the following countries: Ireland (1,977), Denmark (1,515), The Netherlands (1,167), Belgium (818), Cyprus (706), Sweden (649), and Malta (523).

As for the density compared to the arable land available country, the range is as follows (number of pigs per 100 ha): Malta (713), The Netherlands (577), Denmark (520), and Belgium (456) have the highest pig density per ha arable land; whereas the lowest density is registered in Latvia (25.3), Greece (25.5), United Kingdom (30.2), Bulgaria (34.2), and Rumania (35.5).

2.1.3. Poultry production (broilers and laying hens)

In the EU-27, 1.58 Billion head of poultry (= 780 million broilers, 500 million laying hens, and 0.22 million other commercially raised birds such as turkeys, ducks and geese) were raised in 2005. France (283.3 million), Spain (174.4 million), United Kingdom (173.9 million), Poland (151.4 million), Italy (149.1million), Germany (120.6 million), The Netherlands (95.5 million), and Rumania (81.7 million) are the "big" poultry producers, and Luxembourg (0.08 million), Malta (1.0 million), Estonia (2.1 million), Slovenia (3.3 million), Latvia (4.0 million), Cyprus (4.3 million), and Lithuania (9.8 million) are the "small" poultry producers in the EU-27.

As for the flock sizes, there is a clear difference between the EU member states of the "old" EU-15 (until 1995) and the 12 new member states: whereas in the EU-15 "only" 1.12 million poultry operations (all poultry categories) exist, is this number for the new member states 3.28 million operations. These differences are reflected by the fact that the in average biggest holdings (all categories of poultry) by far are found in The Netherlands (31,199 birds per holding), followed by Belgium (6,550 birds per holding), Finland (5,489 birds per holding), Denmark (5,020 birds per holding), and United Kingdom (4,096 birds per holding), whereas the only new membership with more than 1,000 birds per holding is in the Czech Republic.

However, France is with the highest numbers of birds in the country (283.3 million), 163,280 poultry holdings with an average number of birds per holding of 1,735 the most important and leading poultry producer in the EU-27.

2.2. Feed production and consumption (including imports) in the EU-27

Most of the information in this chapter is from FEFAC (European Feed Manufacturers Federation, http://www.fefac.org) and FEDIOL (The EU Federation of the European Bean Crushers, Protein Meals Producers and Vegetable Oil Producers, oil and protein meal industry, http://www.fediol.be/) as presented to the working group.

2.2.1. Total consumption of feed by the major food animal species in EU 27

About 470 million tons of feedstuffs are fed in the EU-27 to the major food animal species (cattle, pigs and poultry). A large amount of feed is produced for fish although specific data is not readily available. This may allow for the assumption that, if the small ruminants, and the minor species such as rabbits, farmed game and so on are added, more than 600 million tons of animal feed is used for the production of animal protein for human consumption in the European Union. Of the total amount of feedstuffs recorded as be used for the major food animal species (470 million tons), about 49% is forage, 30% industrial compound feed (141.7 million tons), 12% is home-grown cereals and 9% purchased straight feedstuffs (feed materials purchased aimed for direct feeding on farm).

2.2.2. Compounded feed production by EU 27 feed industry

In the EU-27 is produced a total of 141.7 million tons of compounded feed out of which about 34% is used for pigs, 31% for poultry, 27% for cattle, 7% for other species and 1% for milk replacers.

The major feed material used (percent of total) are: 47% feed cereals, 27% oil seed residues (cakes and meals), 13% co-products from the food industry, 3% Minerals, Additives, and Vitamins, and several other minor categories such as dried forage, dairy products, oils and fats, pulses, tapioca and others.

The production of compounded feed and the number of feed mills in the MS is rather proportional to the size of their animal food production and the largest producers being France with 21.6%, Germany with 20.0%, Spain with 19.8%, the United Kingdom with 14.2% of the

total production in the EU. In contrast the medium size of feed mills in terms of average production per feed mill is largest in Finland, Sweden and the Netherlands with on average over 120,000 tons yearly production per mill, whereas in e.g. Italy the average production per mill of less than 30,000.

2.2.3. The production and use of oil seed meals in the EU

In the EU-27, about 40 million tons of oil seed were crushed in 2006. The dominating sources of seed are soy beans and rapeseed. Apart from the vegetable oil and other products (mainly lecithin/phospholipids - main use as a lubricant and emulsifier in the food, feed and pharmaceutical industries) produced by the crushing industry for the food market, meal is a protein rich by product used as animal feed.

Approximately 150 production units operate across the European Union. Some plants are located in major seaports and concentrate on one type of seed (some units have an annual crushing capacity of well over 1 million tones of soybeans); other plants carry out processing activities based on the crushing of several type of seeds (soybean and/or rapeseed and/or sunflower), some of which are imported and some produced locally. Other plants depend almost exclusively on raw materials produced locally.

Due to mergers and acquisitions, more than 75% of the European capacity belongs now to a small number of major international groups.

The majority (80%) of the oil seed producers ("crushers") are organised in FEDIOL FEDIOL's members' total crushing capacity is of about 30 million tonnes. The FEDIOL members in the EU-27 use about 14 million tons soybeans, 15 million tons rape seed, 5 million tons sunflower seeds and 2 million tons of other seeds in their production. The majority of oil seed crushing is done in Germany (10 million tons), followed by The Netherlands (4 million tons), France (3 million tons), Spain (3 million tons), Italy (2 million tons), Belgium (2 million tons), and the UK (2 million tons). The European crushing facilities vary in their production capacity from 300 to 6000 tons per day.

Another major player in the industrial production of feed is European association COCERAL which is composed of national trade organisations representing one or more branches of the cereals, rice, animal feed, oilseeds, olive oil, oils and fats and agro-supply trade of the member countries of the EU (http://www.coceral.com).

2.2.4. Imports of feedstuffs and feed ingredients

The contribution of imports to the total amount of compound feedstuffs used in the EU is varying with the type of components. Of the about 278 million tons of cereals (soft wheat, durum wheat, barley, maize etc.) consumed in the EU, only 16 million tons are imported (it can be assumed that the majority of these imports is used for products for human consumption, and that for feeding of animals very little cereals are imported).

Globally there is a great demand for vegetable protein for animal feed. It is generally considered that rapeseed meal, with 37% protein content hardly can substitute soymeal in animal feeding. Rape seed meal can enter feed ratios in the proportion of maximum 15% for chickens and 20% for pigs and dairy cows. As a consequence the EU is currently dependent on importing vegetable protein and soy beans or soy bean meals being the major product. According to FEFAC the self sufficiency in EU 25 is 2% (protein equivalent) for soy bean meal but significantly higher (72%) for rapeseed meal (Table 1). The imported oil seed meal is produced in crushing plants in the exporting countries, mainly in Argentina and Brazil. Soy beans are grown and imported mainly from Brazil and USA (Appendix A, Table I).

Table 1. **EU-25 balance sheet for protein rich feed materials in 2005/2006 (Source FEFAC)**

	EU production (*)		EU consumption (**)		Self-sufficiency (protein equivalent)
	Products	Proteins	Products	Proteins	
Soyabean meal	726	319	34,784	15,305	2%
Sunflower meal	1,988	381	4,503	1,225	31%
Rapeseed meal	8,291	2,079	9,254	2,868	72%
Cottonseed meal	512	179	511	198	90%
Copra-Palm meal	0	0	3,130	501	0%
Pulses	3,350	754	3,850	810	93%
Dried forage	4,600	736	4,400	784	94%
Corn gluten feed	2,193	430	4,550	893	48%
Miscellaneous	376	71	1,047	307	23%
Sub-Total	22,036	4,949	66,029	22,891	22%
Fishmeal	521	370	982	651	57%
Total	**22,557**	**5,319**	**67,011**	**23,542**	**23%**

3. Processing procedures in feed production

3.1. Feed-mills

3.1.1. Handling and storage of ingredients

Major ingredients with a low moisture content are deposited in the feed mills in the intake pit, while minor ingredients are brought into the feed mill by pneumatic transport or in bags. Intake pits are fitted with a conveyer in the bottom part transporting the ingredients to elevators that bring the ingredients to the storage bins inside the feed mill. However, storage of ingredients in large volume occurs most commonly in flat stores in storage buildings associated to the feed mill. Cereal grains, are usually received directly from grain silos associated with the feed mill via conveyers or pneumatic transport systems, and are stored in the feed mill in smaller quantities. Liquid ingredients, such as fat, are pumped to containers or storage tanks associated with the feed mill. Dry ingredients are delivered by trucks, rail cars or by vessels, liquid ingredients by oil tankers. When flat stores are used the transport are usually by means of trucks or by bucket loaders from vessels or rail cars.

3.1.2. Processing of ingredients

The next step in the feed processing is weighing, followed by grinding or mixing of the different ingredients according to the feed formula. In some mills the entire mixture of ingredients is ground for each lot of feed, in other mills the individual ingredient is ground separately. Grinding is performed at ambient temperature and there is usually a slight increase in the temperature of the product after the grinding. Mixing of the ingredients takes place in the mixer where also liquid ingredients e.g. fat may be added. Usually there is only one mixer in a feed mill.

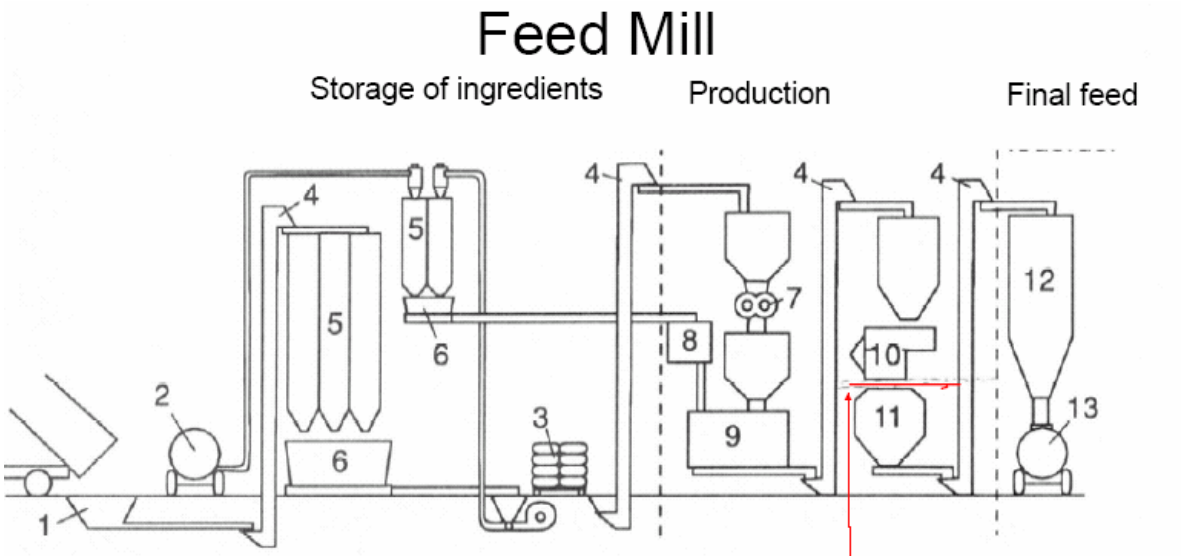
After mixing, the mixture of feed ingredients is transported and stored as meal or mash feed in the finished-product bins, or will be further processed in the conditioning and pelleting process. The size of the mixer will determine the size of the feed lots being produced which is usually 3-5 tons in larger mills.

3.1.3. Conditioning and heat treatment of feed

Conditioning, followed by pelleting or expanding are the usual processing procedures performed in most feed mills when heat treated feed are manufactured. Typically, the meal is introduced into the conditioner where steam is added to raise the temperature to the preset temperature. Pelleting, carried out in the pellet press, involves temperatures between 50° to 90° C. For ruminant rations the temperature during pelleting is usually lower than for pig or poultry feed. The moisture content of the feed after pelleting is approx. 15% and is cooled to ambient temperature and dried to approx 12-13% as rapidly as possible to prevent condensation in the transport equipment and storage containers. Pelleted poultry feed are sometimes sprayed with liquid fat after cooling of the pellets. After processing, the feed is stored a short period of time in silos for compound feedingstuffs before being transported to the farm. The design and construction of the feed plant will to different degrees allow effective physical separation of the clean and non-clean parts of the production likewise permit effective cleaning measures.

The build-up of dust is a factor inherent in feed manufacturing. Therefore adequate dust collector systems in the feed mill are important to control dust and to keep the mill in a clean condition.

Figure 1. **Schematic diagram of a feed-mill**



1. Intake pit for trucks, **2.** Pneumatic intake, **3.**Intake pit for bags, **4.** Elevators, **5.** Storage bins, **6.** Scales, **7.** Mill, **8.** Pre-bin for premixes, vitamins etc., **9.** Mixer, **10.** Conditioner and pellet press, **11.** Pellet cooler, **12.** Storage bin for compound feedingstuffs, **13.** Bulk truck

3.2. On farm mixing (home mixing) of feed.

A substantial proportion of feed produced for animals is home produced. This is highly dependent on the sector of the livestock industry involved, as an example in the UK a survey performed in 2006 [Defra National Statistics, Animal Feed (http://statistics.defra.gov.uk/esg/datasets/hstcomps.xls)] suggested that around 12,000 tonnes of home-mixed poultry rations were produced, which represents approximately 0.5% of total poultry feed production. The major use of home-mixed poultry feed is for commercial egg-laying birds (Richardson, 1971) but home-mixed rations are rarely used for fast-growing meat birds which require a higher nutrient density and well-balanced diet to achieve maximum economic growth and health potential (Schmidt and Zollner, 1931; Goodband *et al.*, 2002).

Large quantities of ingredients are also easier to handle and mix if finely ground then pelleted (Koch, 1996).

It is estimated by the UK Meat and Livestock Commission that around 40% of feed produced for pigs may be home-produced. This includes wet feeding of meals and co-products such as whey. As with poultry, pigs normally grow and breed best when fed purchased pelleted rations (Watson *et al.*, 1978; Hagenbuch, 1982; McIntyre, 1983; Walker, 1987). The cost-benefit of home-mixing depends on the cost of ingredients versus compound feed and consolidation of the industry allows cost savings on investment on materials for feed processing (Anon. 1981; Marbery, 1992; Goldbach and Alban, 2006). Wet feeding in particular may have cost advantages for large finishing units and may also be used in breeding herds. Home-mixed rations are more likely to be used for ruminants where the predominant part of the diet is normally forage and simply cereal protein based blends are added to produce a balanced ration. Cereals do not have to be completely ground to feed ruminants and are often cracked or rolled (Pottier *et al.*, 1996). Beef cattle are often fed on low-grade forage combined with simple protein sources such as urea. In recent years larger dairy herds have increasingly been fed total mixed rations (TMR) in which the forage component is mixed with various cereal and protein based ingredients in a forage-wagon which discharges into feed troughs (Jaworski, 1986; Fournier, 2001). Organic acids such as formic acid may be added to silage or home-mixed rations and this may have some beneficial effect on performance as well as *Salmonella* control (Yli-Hynnila, 1996).

There appears to be very little literature concerning the risk of introduction of pathogens such as *Salmonella* to livestock as a result of home-mixing of contaminated ingredients. These are considered to be a significant source of *Salmonella* for pigs (PHLS Working Group *et al.*, 1972) and poultry (Dougherty, 1976; Crump *et al.*, 2002). In some countries strict precautions are taken to minimise *Salmonella* contamination of commercial compound feed but there is far less control of materials used for home-mixing, which may often be collected direct from docks without any testing or treatment (Martensson *et al.*, 1984). Good storage facilities are needed to avoid spoilage and contamination of feed (Brugger, 1983) and this may be deficient in many situations (Goldbach and Alban, 2006) such that contamination by rodents, wild birds, insects and development of condensation is poorly controlled (Harnisch *et al.*, 1986; Davies *et al.*, 2004). The lack of heat treatment step due to cost (Grimshaw *et al.*, 1975; Gjolberg, 1988; Palkin, 1991) also means that there is no critical control point (Tothi *et al.*, 2002; Davies *et al.*, 2004) although any risk can be mitigated by use of antibacterial additions such as organic acids, which may also have a beneficial effect on general *Salmonella* levels in animals receiving the feed (Creus *et al.*, 2007). Less sophisticated facilities, including mobile equipment which may transfer contamination between premises, and inferior quality control measures are used (Morgan, 1967; Ulvne, 1986; Shurson, 1989; Herrman, 1997; Galey *et al.*, 2000; Kazarinov *et al.*, 2000; Gready, 2005). This means that there may be a significant under-recognised risk associated with home-mixing. On the other hand the bulk buying and prolonged storage of ingredients may lead to a reduced risk of exposure to low frequency contamination events compared with the large and diverse throughput of commercial mills.

3.2.1. Contamination of feed production facilities on farm.

The on-farm milling facility is normally a secondary operation to the main livestock enterprises on farm and is unlikely to employ full-time-staff who only work on feed production. For convenience the milling facilities are normally close to livestock buildings and common vehicles such as tractors may be used in the mill and around the farm, both delivering the feed to various parts of the farm and for other tasks including harvest and livestock manure handling.

When *Salmonella* is present in cattle, pigs, commercial layers or turkeys on farms where home-mixed feed is also milled, there is a significant chance of cross-contamination of storage facilities for grain, other ingredients or compound feedingstuffs by wild birds, rodents, insects, feral cats and other animals whose movement is not controlled. Such contamination may result in a risk of transfer of *Salmonella* to various parts of the farm or to other premises if one site is used for production of feed for others, a common occurrence in small commercial laying companies which may lead to an increased risk of infection (Snow *et al.*, 2007). The extent to which this indigenous feed contamination contributes to cycle of infection on the farm is not known, and the contamination normally fluctuates in parallel with the *Salmonella*-status of the livestock on the site, so may in most cases be more likely to be an indicator of general contamination than a major risk factor.

In conclusion, although standards of feed production and microbiological safety are undoubtedly lower in home-mixing facilities than in compound feed production there are a variety of conflicting potential risk factors, 'gut-health' related and protective factors (Rehman *et al.*, 2007) to be taken into account and more epidemiological work is required to adequately define these risks under current agricultural conditions. This information should be used to assess, quantitatively if possible, the risk of feed-related *Salmonella* serotypes to final consumers (Capita *et al.*, 2007).

4. Microbiological hazard identification and characterisation

A large diversity of feedingstuffs of vegetable or animal origin are used for food-producing animals, either produced at the farm or purchased from feed mills, and conserved under a variety of conditions. This scenario presents different opportunities for contamination with pathogenic bacteria and poses a challenge to achieve adequate levels of feed safety. Available information has demonstrated that different pathogens can be spread by feed in particular *Salmonella* spp. and *Listeria monocytogenes.* Evidence for feed transmission of other pathogens is scarce.

4.1. Salmonella spp.

4.1.1. *Salmonella* in animals

Salmonella infection of livestock has differing manifestations according to the livestock species and *Salmonella* serotype(s) involved. The evidence of surveillance in Europe and elsewhere (Wierup, 1994; Davies *et al.*, 2004; Anon., 2006b; EFSA, 2006a; EFSA, 2007c), and of clinical experience (Taylor, 1989; Eddy, 2004; Jones *et al.*, 2004) is that infections of pigs and poultry are often widespread in many Member States but typically asymptomatic, whilst ruminants may be less frequently infected but more often show clinical signs, also in adult animals. *Salmonella*-associated diarrhoea in pigs is often seen in the context of other endemic pathogens, and *Salmonella* is often a co-isolate with other pathogens from diarrhoeic calves. Certain *Salmonella* serotypes are evidently species-adapted and associated with predictable clinical disease, relevant examples being *S.* Gallinarum and *S.* Pullorum (causing fowl typhoid and pullorum disease respectively in poultry), *S.* Choleraesuis (causing enteritis and septicaemia in pigs), *S.* Abortusovis (causing abortion in sheep) and *S.* Dublin, associated with abortion, enteritis and septicaemia in cattle. Among these, *S.* Dublin remains widespread in European livestock production. It has, unlike most other serotypes, the capacity regularly to establish latent, endemic infection in adult cattle, in certain regions in association with prior liver damage caused usually by liver fluke.

Data on prevalence of *Salmonella* contamination in the animal production varies by animal species, country and detection methods applied as presented e.g. in the 2006 Community

Summary Reports on Trends and Sources of Zoonoses (EFSA, 2007c) and in particular for the pig production also in a previous EFSA opinion (EFSA, 2006b).

4.1.2. Prevalence of *Salmonella* in feed materials.

Most ingredients of both animal and plant origin used as ingredients in compound feed seem to be prone to *Salmonella* contamination. However, prevalence data for *Salmonella* in feed ingredients or compounded feed are usually very difficult to compare between different studies due to differences in sampling and analytical methods applied. It is also not possible to compare prevalence data calutated on the number of *Salmonella*-contaminated samples or on contaminated batches. The different sampling plans used in the monitoring are of particular importance because *Salmonella,* when present in feed, is usually in low numbers and is unevenly distributed, which makes the surveillance sampling critical. In most studies no information is available about the probability to correctly identify a *Salmonella* positive consignment. The lack of information about the surveillance sampling will also raise questions about the confidence in negative results in the different studies.

Despite the sampling uncertainties ingredients used for animal feedingstuffs have been shown in several studies to commonly be contaminated with *Salmonella* (Hacking *et al.*, 1978; Kidd *et al.*, 2002; Jones and Richardson, 2004; Dargatz *et al.*, 2005; Anon., 2006b; Anon., 2007a,b; EFSA, 2006a; EFSA, 2007c). The prevalence of *Salmonella* in different feed ingredients can be summarized as follows:

4.1.2.1. Animal-derived protein.

In response to the need to prevent the spread of BSE a total ban of feeding processed animal protein in feeds for any animal farmed for the production of food was introduced 1 January 2001. Later some exceptions have been introduced such as the use of fish meal and certain blood products and dicalshiumphosphate (by-products e.g. from production of gelatine) as feed for non ruminants as described in the current legislation (EC No. 1292/2005, http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_205/l_20520050806en00030011.pdf). A strategic goal is a further relaxation of certain measures of current feed ban when certain conditions are met. Further improvement in differentiating animal protein specific to certain species may result in an amendment of the provision with regard to the use in feedingstuffs of animal products, in particular non ruminant protein taking into account the prohibition on intra-species recycling regulation (EC) No 1774/2002, http://eur-lex.europa.eu/LexUriServ/site/en/consleg/2002/R/02002R1774-20070101-en.pdf (e.g. poultry meat and bone meal/MBM to pigs). The OIE is recommending principally the same feed restrictions as in the EU to be applied for international trade but a ban on feeding MBM is not applied for the domestic market in several third countries. Currently there are principally no specific restrictions with regard to the use of tallow in feed or food to prevent transmission of TSEs or for dairy by- products.

When allowed as ingredients of animal feed, mammalian MBM and poultry offal meal were found to be frequently contaminated by *Salmonella,* a logical consequence of the risk from the rendering of animals infected with *Salmonella,* some of which could be clinical cases (Thal *et al.*, 1957; Hirsch and Sapiro-Hirsch, 1958; Knox *et al.*, 1963). Several examples also illustrate that products from the rendering industry are more frequently contaminated with *Salmonella* than other ingredients. In a series of publications since 1958 data on *Salmonella* contamination in imported feed of animal origin as well as in the domestic production of meat-meal and compound feed were published from investigations carried out in Sweden (Rutqvist and Thal, 1958; Karlsson *et al.*, 1963; Hurvell *et al.*, 1969; Gunnarsson *et al.*, 1971; Sandstedt *et al.*, 1980; Martensson *et al.*, 1984; Eld *et al.*, 1991; Malmqvist *et al.*, 1995). These data

demonstrate that *Salmonella* has been frequently isolated from feed raw materials particularly of animal origin as long as 50 years ago. Also in the 2005 zoonoses report (EFSA, 2006a), where data of *Salmonella* in animal derived feed materials are compiled, essentially all countries annually report *Salmonella* from MBM. The risk for the *Salmonella* contamination is also found to be attributed to in-house contamination in the rendering plants and recontamination following the heat treatment process. Monitoring by the EU feed industry also verifies that animal meal frequently is contaminated by *Salmonella*; during 2005 14.9% of 94 samples were contaminated and 8.3% of 72 samples during 2006 (Anon., 2007a). The increased standards of heat treatment of animal by-products following TSE legislation led to a reduction in *Salmonella* contamination, but despite this UK reports suggest around 2% of batches testing positive in 2006 (Anon., 2007b). This is thought to be largely caused by post-processing contamination from dust, contaminated equipment or leaking cooker seals.

Fish meal also has the potential for the spread of *Salmonella* although fishmeal seems to be somewhat less contaminated than other animal derived protein feed according to the EFSA zoonoses report from 2005. As for MBM the risk of *Salmonella* contamination is found to be attributed in-house infection in the rendering plants and recontamination following the heat treatment process. *Salmonella* contaminated fish meal was the source to the most well known example of feedborne transmission of *Salmonella*, when S. Agona emerged as a public health problem in several countries due to contaminated imported fish meal. In the United States a rapid increase of human infections with S. Agona occurred from 1968 to 1972 (Clark *et al.*, 1973). Similarly, human infections with S. Agona occurred simultaneously in European countries. Since then, S. Agona is among the most prevalent serotypes in humans in the USA alone and it is estimated that the serotype has caused more that one million human illnesses in the USA alone since it was introduced into the food chain (Crump *et al.*, 2002).

There is a potential risk for the spread of *Salmonella* by feeding animals also by some dairy by-products (in particular raw milk, non-pasteurised white water and whey from raw / unpasteurised milk cheese processing) as highlighted in a previous EFSA opinion (EFSA, 2006c). Product Board Animal Feed (Anon., 2007a) data from 2005 and 2006 indicate a low prevalence in whey for when 0 and 0.2% respectively of samples were found *Salmonella* coontaminated.

In summary, animal-derived protein is considered as a high risk product for *Salmonella* and an additional risk exists also for the spread of epizootic diseases. This is also reflected in the legislation (Regulation (EC) No 1774/2002 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:273:0001:0095:EN:PDF), which has a requirement for freedom from *Salmonella* in 25 g of such products. However, that risk is currently limited as a result of the EU- ban on the feeding of mammalian and avian derived proteins but may exist in third countries and recur in the EU if in the future such products again are allowed as animal feed.

4.1.2.2. Vegetable protein.

(i) Non-processed products.

Data from non-processed products are scarce. However, *Salmonella* were isolated from approximately 30% (12- 68%) of samples tested from dust of all lots of soy beans imported mainly from South America to Norway during 1994-2007 (Denofa, 2007); Appendix A. Figures I and II).

(ii) Processed products.

Several examples also illustrate that products from the crushing industry are often contaminated with *Salmonella*. As an example, in a larger Dutch report feed materials from 2002 and 2003, 3.2% and 6.7% respectively of Brazilian extracted soy beans were positive for *Salmonella*

(Anon., 2004). In Sweden 14.6% of imported consignments of soy meal were found to be contaminated by *Salmonella* during 2004-2005 and when considering only imports mostly from South America the level was approximately doubled (Wierup, 2006). That level has regularly been found in the Swedish feed control where all consignments are tested before introduction to the feed mills (Häggblom, 1994). Denmark also reports of problems associated to *Salmonella* contaminated vegetable protein, primarily imported soy meal. The competent authority in 2002 reported a two-fold increase in the isolation of *Salmonella* in the process control at feed mills which was considered to be associated to imported *Salmonella* contaminated soy meal (http://www.pdir.dk), and a study during 2004-2005 of imported ship loads of soy meal verified that risk for *Salmonella* contamination (Wierup, 2006). A significantly higher prevalence of *Salmonella* was found in Sweden in the weekly monitoring of feed mills using contaminated soy meal in contrast to those supplied by a safer source. In spite the application of a decontamination procedure using organic acids of batches found to be *Salmonella*-contaminated, the contamination was sometimes also found on the clean side, in final feed and in subsequent infection of swine herds (Wierup, 2006). Available data from the EFSA zoonoses report 2005 also support oil seeds e.g. soy bean products, as a risk factor for introducing *Salmonella* into the feed chain (EFSA, 2006a).

Major sources of vegetable protein for animal feed are also rape seed and palm kernel. *Salmonella* is also frequently reported from these products. Product Board Animal Feed (Anon., 2007a) reports a contamination rate for rape seed meal and flakes of 6.8 and 3.4% for the years 2005 and 2006 respectively (number of samples: 4,378 in 2005 and 4,337 in 2006). In a Dutch monitoring study, 12% extracted rape seed meal were positive in 2002, and 7% in 2003 (Anon., 2004). Experiences indicate a lower prevalence of contamination in these products than in soy meal and in a Swedish study 10% of rape meal batches and 9% of cornmeal batches were during a two year period (2004-2005) found to be *Salmonella* contaminated (Appendix A, Fig III; (Wierup, 2006). UK data records an improving trend in the contamination of oilseed meals and products from 3.3% in 1999 to 1.7% in 2006 (Anon., 2007b).

4.1.2.3. Grain

Grain is seldom found to be contaminated unless as a result of contamination during storage and transport. However, studies show that grain can also be contaminated, especially if it originates from areas where *Salmonella* is common in wildlife or local livestock. It is common to find *Salmonella* serotypes which are associated with wildlife or local livestock in dust collected from grain drying and handling systems and grain storage bins in compound feed mills (Davies and Wray, 1997). S. Typhimurim may also be found disproportionately in wheat and barley compared with other feed ingredients. Some countries report *Salmonella* at a low prevalence in wheat while in other countries cereals seem to be virtually free from *Salmonella*. The latter situation was found in Sweden in studies prior to the introduction of whole wheat as poultry feed in the 1990s. In the zoonoses report from 2005 data for *Salmonella* in animal derived feed materials are compiled from different countries and cereals generally seem to be less contaminated than processed animal or plant protein. UK data suggests a 0.3% contamination rate of grain in 2006 (Anon., 2007b). However, it is possible that the contamination of grain may be underestimated because of the relatively small surface area of the material tested (Jones and Ricke, 1994) compared with vegetable oil-seed residue meals.

4.1.2.4. Forage

Data on *Salmonella* contamination of forage seem to be very scarce but generally forage feed is not associated to risk for contamination with *Salmonella* unless in exceptional cases.

4.1.2.5. Conclusions in relation to the risk of *Salmonella* contamination.

Due to the fact that the risk for *Salmonella* contamination is found to vary between different feed materials and country of origin it is suggested that the prevention and control of such contamination is related to that risk.

4.1.3. Prevalence of *Salmonella* in compound feed

Contamination of compounded feed by *Salmonella* is not uncommon even in feed that have undergone heat treatment (Hacking *et al.*, 1978; Cox *et al.*, 1983; Veldman *et al.*, 1995; Österberg *et al.*, 2006). Recent data from EU member states shows national prevalences for compounded poultry feed of 6% for some countries, with most countries are in the range from 0% to 1.5% (EFSA, 2006a). Similar contamination rates were reported for pig (up to 1.7%) and cattle (up to 4%) feeds in the EU. In MS with a low prevalence of *Salmonella* in food producing animals *Salmonella* is only occasionally found in compounded feed. The industry based data from 2005 and 2006 (Anon., 2007a) reports an incidence between 0 and 0.8% of *Salmonella* contaminated samples in compounded feed to different food animal species (poultry, swine and cattle).The lowest prevalence was found in feed for top breeding poultry flocks and the highest for laying hens. UK data from 2006 reports a 0.4% contamination rate of pelleted poultry feed and 0.6% for pig and poultry meals (Anon., 2007b). It is difficult to interpret such data however as sampling plans are highly variable and include data both from routine surveillance and specific trace-back or HACCP based investigations so no true prevalence data for UK exists.

Incidence of *Salmonella* in feed has been studied in a Spanish surveillance program in feed developed during 2007. A total of 700 feed mills were visited, with 2.100 feed materials and 2.100 compound feed batches sampled. Preliminary results from 308 feed mills showed a 3.5% incidence in feed materials and 3.5% incidence in compound feed (for all *Salmonella* serotypes) (Sobrino, 2008).

Surveys of the prevalence of *Salmonella* in feed mills have shown that *Salmonella* was frequently recovered from the pre-heating as well as from the post-heating treatment areas of the mill. High *Salmonella* prevalence was also detected in dust samples from the post-heating treatment area of the mill, feed delivery vehicles, as well as inside the pellet cooling systems (Davies and Wray, 1997; Whyte *et al.*, 2003).

4.1.4. Considerations on *Salmonella* serotypes found in feedingstuffs

Subtyping of isolated strains of *Salmonella* into serotypes, as well as other methods for further sub classification, are important and necessary tools for tracing the sources to *Salmonella* contamination of the feed and food chain and of subsequent infections of animals and humans. The classification of *Salmonella* to the level of serotypes has revealed the substantial risk for the feed production to be exposed to different serotypes of *Salmonella*. As an example 77 different serotypes of *Salmonella* were identified during the period 1994 -2007 from dust of soy beans imported mainly from South America to Norway (Appendix A. Figure I) and in Sweden 31 different serotypes were isolated from different sources of vegetable protein during a two year period 2004-2005 (Wierup, 2006). The prevalence of a wide range of serotypes in animal feed ingredients and compounded feed is not a recent event, and has been observed since monitoring began e.g. in early studies from Sweden (Rutqvist and Thal, 1958; Karlsson *et al.*, 1963; Hurvell *et al.*, 1969; Gunnarsson *et al.*, 1971; Sandstedt *et al.*, 1980; Martensson *et al.*, 1984; Eld *et al.*, 1991; Malmqvist *et al.*, 1995). The primary source for the multiplicity of different serotypes identified in vegetable protein is not known and would merit further studies.

In the 2005 Community Summary Report on Trends and Sources of Zoonoses (EFSA, 2006a) the dominant serotypes encountered in samples of primarily compounded feed were *S.*

Livingstone, *S.* Senftenberg and *S.* Montevideo. The isolation of *S.* Enteritidis, a, major serotype in human salmonellosis, occurs only occasionally [except perhaps in Japan (Shirota *et al.*, 2000)], whilst *S.* Typhimurium appears to be a more widespread contaminant, albeit not a dominant one (Malmqvist *et al.*, 1995; Anon., 2006b; EFSA, 2006a). UK data from 2005/2006 (Anon., 2007b) reports the greatest diversity in serovars in vegetable oil residue meals and similar serovars, such as *S.* Agona, *S.* Rissen and *S.* Senftenberg are most likely to be found in compound rations, although this is also influenced by resident contamination of some feed mills with serovars such as *S.* Ohio and *S.* Kedougou. *S.* Typhimurium is occasionally found and is more likely to be associated with grain, whereas *S.* Enteritidis is now rarely isolated from feed since improved control of this serovar in poultry. In the monitoring of *Salmonella* in the animal feed sector (Anon., 2007a) five *Salmonella* serotypes (Enteritidis, Typhimurium, Infantis, Virchow and Hadar) are classified as critical. During 2006 the frequency of the isolated serotypes being critical were as follows for different feed materials: South American soya meal as well as rape seed meal and flakes both 20%, soya beans toasted 11%, European sunflower meal 50%, while fish meal, egg shells and French wheat bran all 0%.

The repeated and long term isolation of certain serotypes in feed ingredients or compounded feed have often been found to be the result of persistent contamination of crushing and feed producing plants.

Although only a minority of the serotypes isolated from animal feed is found to cause clinical disorders in animals, they may all be pathogenic to humans. However, contamination of feed with those serotypes being pathogenic or adapted to certain animal species (e.g. *S.* Typhimurium and *S.* Enteritidis), usually result in intestinal colonisation, and a long term shedding and subsequently to a persistence of the infection at the farm level, with an additional risk for a further spread of the infection to other animal holdings, environment and to humans.

However, serotypes primarily found to be of low virulence to animals may be adapted to a certain species like e.g. *S.* Derby which in different countries is found to be adapted to swine with a subsequent spread in the swine production (Wierup, 1994). A more striking example is the pandemic of S. Enteritidis phage type 4 during the 1980s through a change in the virulence of the microbe which since 1990s has become a major cause of human infection (Sobel *et al.*, 2000). A recent example of the establishment of a virulent serotype in the food chain was observed in 2007-2008 when *Salmonella* Reading was isolated for the first time in humans in Sweden where ground beef was the source of infection. Two herds of cattle and pigs were found to be positive for this new serotype. Subtyping of the isolated strains has shown that all isolated strains are identical supporting a common source of infection. *S.* Reading has also been isolated from imported soybean meal in one feed mill. The epidemiological investigations have so far not shown any common source of infection for the animals involved. *S.* Reading had previously spread in pigs in the UK, as well as being occasionally isolated from feed (Anon., 2007b).

In summary, the risk posed to animal health as a result of feed contaminated with *Salmonella* is principally the same as the risk posed to human health as a result of *Salmonella* contaminated food, although the infectivity for different animal species and humans may vary by serotype of *Salmonella*.

4.2. Listeria monocytogenes

Listeria monocytogenes is common in soil, sewage, forage and water and consequently can be present in different vegetation. In the E.U., in 2005, *Listeria monocytogenes* was occasionally reported from various animal species, showing that animals are one source of *Listeria monocytogenes* contamination. Some of those animals (sheep and goats) related to clinical samples, but probably most of these animals were asymptomatic intestinal carriers, shedding the organism in significant numbers, contaminating the surroundings (EFSA, 2006a).

Listeria spp. have been found in poultry feeds both before and after the heat treatment (Blank *et al.*, 1996; Whyte *et al.*, 2003). In this study (Whyte *et al.*, 2003)it was noted that much of the environment in the feed mill was contaminated with *Listeria* spp which could suggest that recontamination of pelleted feed may occur.

The prevalence of *Listeria monocytogenes* in animal feed having a low level of available water (hay, cereal grains) is very low and the numbers are probably unlikely to reach levels that present a serious risk to animals (Fenlon, 1999). Wet feeding of pigs during the fattening period was identified as a risk factor for *Listeria monocytogenes* (Beloeil *et al.*, 2003). Furthermore, it is well known that the risk of the presence of *Listeria monocytogenes* in silage is related to animal listeriosis and asymptomatic carriage, mainly in dairy cattle, sheep and goats (Skovgaard and Morgen, 1988; Nightingale *et al.*, 2004; Wagner *et al.*, 2005). The link between aerobically spoiled silage and cases of listeriosis in farm animals has been described; in addition the risk of the presence of *Listeria monocytogenes* in raw milk is higher when cows are fed with silage with pH 4 and above. Consequently it is important to produce silage anaerobically and controlling the pH (less than 4) to avoid the growth of *Listeria monocytogenes* and the subsequent contamination of animals and introduction into the food chain.

4.3. Escherichia coli O157

Escherichia coli O157:H7 has rarely been detected in cattle feed. However, recent reports suggest that feed may be a source of *E. coli* O157 in cattle (Dodd *et al.*, 2003; Sargeant *et al.*, 2004; Dargatz *et al.*, 2005). 0.5% of purchased feed stored at the farm were positive for *E. coli* O157 (Hancock *et al.*, 2001) Reports have also shown that *E. coli* O157 may multiply in some cattle feeds where there is sufficient water content (Lynn *et al.*, 1998; Fenlon and Wilson, 2000). In a recent paper, it is concluded that the time / temperature combinations used in commercial pelleting processes do not effectively kill high numbers of *E. coli* O157 present in the feed (Hutchinson *et al.*, 2007).

4.4. Clostridium spp.

C. perfringens is an obligate spore-forming anaerobe, common in faeces (Tschirdewahn *et al.*, 1991) and soil (del Mar Gamboa *et al.*, 2005). It therefore is a common component of feedstuffs, either as vegetative cells or hardy, thermoresistant, endospores (Xylouri, 1997), and is particularly prevalent in soil-contaminated feeds such as root crops (Secasiu, 1982). However, among feedstuffs generally, animal protein sources and compounded feeds usually have the higher frequency of contamination (Kaić, 1977; Chakrabarty and Boro, 1981; Xylouri, 1997), with a higher the concentration of *C. perfringens* (Wojdat, 2006), and a higher prevalence of toxigenic strains (Secasiu, 1982; Wojdat, 2006). Prió *et al.* (Prió, 2001) reported that there was little or no correlation between levels of clostridial contamination in raw ingredients and in compounded feeds (in either meal or pelleted form) derived from them. By contrast, the degree of *Salmonella* contamination in ingredients was positively correlated with that in compounded meal, and additionally there was effective suppression only of *Salmonella* contamination by pelleting. Clostridial contamination is not effectively controlled by the heat and pressure of conventional pelleting, and additional contamination may also readily be acquired during the compounding process.

In view of the common isolation of *C. perfringens* from the environment and from the intestinal tracts of livestock (75% to 95% of broilers) (van Immerseel, 2004a), and the fact that *C. perfringens*-associated diseases appear to need initiators in addition to the presence of the organism (Songer, 1996; Craven, 2000; van Immerseel, 2004a), the significance of feed contamination by this bacterium is open to question. Feed has however been implicated in some fowl necrotic enteritis outbreaks (Frame and Bickford, 1986; Dosoky, 1990), and in one

study of four pig farms, the unit having toxigenic *C. perfringens* in sow feed had the highest mortality for necrotic enteritis in piglets (Udovičić, 1994).

Botulism in animals has been reported in many countries, and over many years. Most clinical cases of *Clostridium botulinum* intoxications are related to equines and cattle (Galey *et al.*, 2000; Kelch *et al.*, 2000). The common source of the toxins is silage or haylage of poor quality, particularly under conditions when the grass has wilted or been spoiled. Spreading on to pasture of contaminated poultry litter containing sometimes dead poultry, can be a source of contamination of cattle (Anon., 2002). Outbreaks of botulism have also occurred in poultry flocks and poultry litter may be a source of *C. botulinum* (Livesey *et al.*, 2004), mainly when dead poultry carcasses remain on the litter (Blandford and Roberts, 1970; Harrigan, 1980). A large outbreak of type C botulism in fur animals occurred recently where the deaths could be associated with feed manufactured by a local processor (Lindström *et al.*, 2004). Although the source can sometimes be difficult to find, in general, the cause of botulism in animal is the multiplication of and the toxin production by *C. botulinum* in the feed consumed. As an example, in France, *C. botulinum* type A (Gimenez and Ciccarelli, 1987), type C (Dohms *et al.*, 1982) and D (Popoff, 1989) were present in feed sampled at the farm where outbreaks occurred. Nevertheless the detection of the presence of the toxin in the feed is often very difficult (Anon., 2002).

4.5. Campylobacter spp.

No published data were found in the literature search indicating that commercial feed is a source of campylobacter infection in food-producing animals and this is unlikely because of the dry conditions and exposure to air involved in feed production.

4.6. Antimicrobial resistance in bacterial contaminants of animal feedstuffs.

There appears to be ample potential for the introduction of antimicrobial-resistant bacteria to animal production units by feedstuffs, given the limitations of conventional feedmill treatments in eliminating common bacterial contaminants (da Costa *et al.*, 2007). The subsequent risk of increasing resistances on-farm and beyond relates not only to successful colonisation by resistant strains of feed origin, but also to the potential for the dissemination of mobile resistance elements to other bacteria, which may be established endemic strains and/or possibly more pathogenic ones. Integron sequences, indicating the capacity to accept and transmit antimicrobial resistance gene cassettes (Hall and Collis, 1998), are commonly found amongst Gram-negative bacteria isolated from rendered animal feedstuffs, in association with widespread antimicrobial resistances (Hofacre *et al.*, 2001).

The genetic linkage of virulence genes with genes coding for antimicrobial resistance on mobile genetic elements emphasises the importance of avoiding international dissemination of resistant organisms in feed (Chu and Chiu, 2006).

In view of the evidence for the importance of enteric colonisation by resistant bacteria and for horizontal dissemination of resistance genes, even in the absence of selective antimicrobial use, the current scattered and sparse data on antimicrobial resistances in feedstuff bacteria should be considered inadequate. A particular concern is the potential for international dissemination in feed or ingredients of plasmids in *E. coli* carrying a variety of genetic mechanisms which confer resistance to third generation cephalospororins as part of a package of multiple resistance genes.

4.7. Conclusion (main hazards / priority list)

By far, the most important bacterial pathogen in feed is *Salmonella* which frequently occurs in a large number of feed ingredients of animal or plant origin and also in compounded feed.

The primary sources of contamination of these products seem to vary. Plant and animal derived proteins are, in several studies, shown to be more frequently contaminated with *Salmonella* than non-processed plant materials. Several of the *Salmonella*-positive feed ingredients of both animal or plant origin are produced in industrial processes where *Salmonella* is destroyed due to the high processing temperatures which indicates that recontamination of the products occurs. Some data shows that e.g. soybeans often are contaminated when they enter the crushing plant, however with the application of strict hygiene rules it has been shown that the produced soybean meal in *Salmonella* negative. By application of hygiene rules it is also possible to produce *Salmonella* negative feedingstuffs in feed mills (Chapter 8.4). Data from studies of *Salmonella* prevalence in forage, home-grown cereals and purchased straight feeding stuffs are scarce.

Evidence for feed transmission of *Salmonella* to food-producing animals such as poultry, pigs and cattle has been presented in several studies. Some of the serotypes detected in feed are more frequently isolated from food producing animals than other.

Other pathogenic bacteria with relevance for animal and human health and where feed might be a vector for the dissemination of the pathogen is limited to a few other species. *Listeria monocytogenes* may be present in all kinds of feedingstuffs but the problems with Listeria seem to be primarily limited to the occurrence in silage of poor quality. *Escherichia coli* O157:H7 has been detected in cattle feed and feed has been suggested to be the source of infection in cattle. No information is available whether strains of *E. coli* O157 isolated from feed cause human disease. *Clostridium botulinum* present in poor quality silage may cause serious intoxications in equines or bovines and *Cl. perfringens* is a known pathogen causing clostridial enteric disease in animals. *Cl. perfringens* is commonly isolated from several animal feed ingredients of animal or plant origin.

It is desirable to include all feedstuffs for food animals into the microbiological risk assessment for feed. However, a risk based approach support a limitation of this report primarily to industrial compound feed. In addition too little is known about forage and home-grown cereals. Thus, all considerations of this report are focussed on the industrial compounded feed including the major risk ingredients in industrial compound feed: the protein rich vegetable protein and animal derived protein.

5. Feed and Feeding systems for major farm animal species including poultry

There is a wide variety of practices for feeding the different animal species and in different intensities of production systems. The following is a brief summary intended to highlight areas of possible relevance to bacterial contamination. For example, poorly-made bagged silage which incorporates soil may result in a risk of multiplication of *Listeria monocytogenes* and accidental inclusion of wild or feral animal tissue may introduce *Clostridium botulinum,* which may subsequently multiply and produce toxins.

5.1. Sheep and Goats

For most of the year these typically graze on grass or forage root or brassica crops. In winter and during pregnancy grazing or silage/hay rations may be supplemented with mineralised rolled grain or simple concentrates. During the lambing period sheep are often housed and fed hay and concentrates in simple troughs, which may also be used when feeding outdoor sheep or, alternatively, feed may be fed on the ground. Some types of indoor feed troughs may also be used as walkways by farmers. Sheep and goat herds used for milk or intensively reared lambs are likely to be housed for a greater proportion of time and fed higher levels of

concentrates. Open feeding systems are attractive to wild birds and may result in feed and feed troughs being contaminated with wild bird faeces.

5.2. Beef Suckler Cattle

Adult breeding herds are normally grazed during the period of the year when grass is growing but housed in winter, although some herds in hill areas may not be housed (Buchanan-Smith and Fox, 1999). Supplementation is unusual in summer but mineral licks may be provided. Suckled calves do not normally receive supplement during the grazing period but may have separate 'creep' feeding areas (Ritchie, 1987) when housed or at pasture. Outwintered cattle are normally fed additional silage, hay or straw (+/- urea), sometimes with additional concentrates which are usually fed on the ground. Similar risks apply to those described for sheep and goats. There is also a risk associated with spreading manure, slurry or sewage sludge, or overflowing contaminated watercourses leading to *Salmonella* infections or, in the case of spreading broiler litter containing carcasses, *Cl. botulinum*.

5.3. Dairy Cattle

There are numerous systems for feeding dairy cattle (Strzetelski and Borowiec, 1998) but in most countries a large part of the diet comprises grazed or zero-grazed grass crops and conserved forage based on grass, maize, lucerne or immature cereal crops (Nird, 1986; Chamberlain and Wilkinson, 1996). Forage may be 'self-fed' at the silage face or cut and carted to the cattle. Brassicas such as kale or root crops such as fodder-beet or potatoes may also be fed. During the grazing period conditions similar to beef cattle apply, but in the case of milking cows there may be additional concentrate feeding either in individual troughs in the milking parlour, sometimes via automated dispensing systems triggered by transponders attached to the cows (Artmann and Schlunsen, 1987), or feeding of concentrate or forage-cereal mixes (Total Mixed Rations [TMR]) in troughs in feeding areas (Hoden and Giger, 1984). These rations are mixed by mobile mixer trailers which may move between farms, which involves a biosecurity hazard. During the winter period the proportion of concentrate rations is increased and there may be more use of 'straights', i.e. purchased vegetable proteins such as soya bean meal, rape-seed meal, maize gluten or palm kernel meal, which may be regularly contaminated with *Salmonella*. However, dairy cattle as well as other ruminants are often fed concentrates in a pelleted form in order to stimulate greater feed intake and increase feed conversion. As the pelleting process is combined with heat treatment that reduces or eliminate *Salmonella* contamination, such feeding practices are likely to reduce the risk of contamination originating from high risk products such as soy bean meal, in contrast to animals fed non-pelleted feed. Poor storage conditions may facilitate a rapid short-term increase in *Salmonella*, before this is overwhelmed by the growth of competitor organisms. Housing and feeding systems as well as flat stores for straight ingredients are often open to intrusion by wild birds, rodents, cats, dogs, foxes and badgers which may defecate in the feed. Maize silage is also very attractive to badgers, (Reilly and Courtenay, 2007) which are the main source of S. Agama contamination of domestically produced feed ingredients (Wray et al., 1977; Wilson et al., 2003).

Dairy youngstock are typically fed a similar ration to beef cattle, including greater use of barley straw and less concentrate. Weaned calves may utilise more purchased concentrate in the early stages, or throughout if they are reared for intensive 'barley beef.

5.4. Calf Rearing

Early weaned dairy calves intended for beef, dairy replacements or veal production normally stay with the dam for 24-48 hrs and are then fed milk replacer based or vegetable proteins such as soya and vegetable fats and carbohydrates (Davis and Drackley, 1998). Contamination of the

powdered milk replacer is not uncommon and the unhygienic conditions associated with bucket feeding, group feeding via teats (Hepola, 2003), or by automated feeders (Hannus and Hanninen, 2001) may contribute to the spread of micro-organisms. Feeding of surplus pooled colostrum, which may be fermented and stored, is also common. Forage may be provided in the form of barley straw bedding, some of which is eaten, or in some cases hay, but this is more expensive. Beef calves or dairy replacement animals are normally weaned onto concentrate rations at around six weeks of age but veal calves will continue on a milk based diet fed by automated feeder or bucket until slaughter.

5.5. Pigs

Pig rations are largely based on concentrates, typically wheat as this provides the lowest cost per unit of metabolisable energy. Proteins are supplied largely from vegetable sources, especially soya bean meal. Outdoor pigs often have access to grass or cereal stubble, but this does not contribute significantly to their nutrition.

Adult outdoor breeding pigs are normally fed large rolls or cobs on the ground. Such large pelleted rations cannot be effectively heat treated at the mill as this adversely affects pellet quality. The feed is distributed over the paddock by a feeding wagon and in wet conditions the feed sinks in contaminated mud, which is also ingested and contributes to the *Salmonella* burden in outdoor pigs. Groups of gilts or lactating sows may receive feed in open ad-lib feeders. These outdoor feeding arrangements are very attractive to wild birds which move between local pig farms following the feeding cycles. Pigs in outdoor weaner and grower kennels are normally fed on small pellets delivered into ad-lib hoppers in the rear of the kennels by hand. These areas often harbour significant mouse or rat populations. Effective cleaning of feeding equipment between batches of pigs is difficult and requires special procedures, and contaminated feeding equipment is a significant means of perpetuating *Salmonella* between batches of pigs.

Adult housed pigs are normally fed medium sized pellets in troughs or on the floor of the pens. In large dry sow yards automated feeders trigged by transponders attached to the pigs may be used (Meunier-Salaun *et al.*, 2002) and most feeding systems on large units are automated. Some units feed liquid feed based on meal feed mixed with water or whey (Cumby, 1986) and some farms with home mixing systems may feed meals to all stock except young pre-weaned and weaned pigs which are typically fed highly palatable small pellets, usually in ad-lib hopper systems, once they have been weaned. In the grower and finishing stages automatically filled troughs or larger ad-lib feed hoppers are commonly used.

Wet feed may be fed ‘fresh’ - directly after mixing, or fermented after a storage period at ambient temperature (for further information on liquid feeding see also EFSA opinion) (EFSA, 2007d). As mentioned elsewhere in this document liquid feeding, particularly when whey is fed, or where fermentation is controlled using a starter culture and controlled temperature conditions, may reduce the risk of intestinal carriage of *Salmonella* compared with pelleted feed, as may reducing the proportion of wheat in the diet in favour of barley, and coarse grinding to slow down fermentation of the feed in the gut.

Pig feeding systems with their complex of troughs, hoppers and pipes have been shown to be particularly difficult to clean and disinfect effectively because of inaccessible surfaces and pooling of wash water. This contamination may be responsible for carry-over of *Salmonella* between batches of pigs.

5.6. Poultry Breeding and Poultry Meat Flocks

There is a large number of different feeding systems in use (Karunajeewa, 1987; Leeson and Summers, 1999). Until recently an open chain feeder system was most common with pelleted

or meal feed delivered from a bulk feed hopper outside the house into a slave hopper with motor unit which distributes feed around a network of metal troughs by means of a moving chain (Smidt and Eiciene, 1999). The movement of feed in an open trough is likely to overcome attempts to limit the spread of infection by segregating groups of animals of enteric infections such as *Salmonella.* Although chain feeder systems appear to be difficult to clean, clearance of *Salmonella* is aided by the oxidation which occurs when they are washed, or disinfected with acidic disinfectants.

Most modern poultry houses have a pan feeder system in which small plastic or metal feed pans (troughs) or tubular feeders are either incorporated in the feed line or larger tube-feeders are fed by separate feed pipes emanating from main feed pipes. The former system is most common in broiler units and can give rise to *Salmonella* contamination problems as the interior of the pipes is not accessible for cleaning and disinfection and if high levels of *Salmonella* have been introduced in feed, or via contamination of in-house slave feed hoppers by dust or rodent faeces, a persistent contamination may occur within the pipes. This has been a notable feature of *Salmonella* Paratyphi B *var* Java infection in broiler flocks in the Netherlands leading to infection in consecutive flocks despite good cleaning and disinfection of the rest of the house (van Pelt *et al.*, 2003). This problem has sometimes been addressed by circulating formaldehyde-treated wood chips to abrade the contaminated aggregate within the pipes and decontaminate the surfaces. A similar approach has been used to decontaminate feeding systems after a *Salmonella* incident. In this case whole wheat treated with a commercial formaldehyde / propionic acid / terpene product is circulated. Some older style turkey houses use large wooden bulk ad-lib hoppers within the house. These are filled regularly by a feed delivery lorry via sloping pipes from the exterior of the building. These hoppers offer very attractive harbourage for rodents both within the hoppers and by burrowing into litter or earth floors below. Accumulated feed in the corners of the trough of the hopper also often becomes moist and low levels of *Salmonella* in the feed may then multiply.

Another system which is sometimes used in chicken breeder rearing flocks is to floor-feed on to the litter using feed distributed by a spinner. Such floor feeding may accentuate the spread of intestinal pathogens if they are present. In summary the feeder systems as well as other equipment and the building should be constructed to allow, cleaning and disinfection so that it can be ensured that a possible *Salmonella* contamination can be eliminated and not transmitted between batches of animals.

5.7. Commercial Laying Flocks

Barn and free-range flocks and rearing flocks tend to be fed bulk feed by similar sorts of systems to those used for meat poultry flocks. Cage layers are fed via troughs attached to the front of the cages and feed is either carried along the length of the house by a spiral auger mechanism in the base of the trough or by moving automated or manual hoppers, which pass along the cage rows dispensing feed as they go. Commercial layers are normally fed coarsely ground meal based on wheat and vegetable proteins since pellets lead to too rapid feed consumption and hunger-related welfare problems, more moist faeces which contaminate egg belts and eggs, and the fine grinding needed to ensure good pellet quality enhances colonisation by *Salmonella* which may be endemic on the farm and makes distribution of limestone flour very difficult. In organic production synthetic methionine and lysine are not permitted and this creates problems in terms of over-supply of total protein in order to achieve adequate levels of these amino acids from natural ingredients. Supplements such as oyster shell are also often fed to enhance the quality of egg shells (Henuk and Dingle, 2002). Outdoor laying flocks are sometimes co-grazed with sheep or cattle, which may present a risk of introduction of *Salmonella*, e.g. *S.* Typhimurium.

The subsidiary mechanised hoppers (slave hoppers) in non-cage units and the whole feeding system in cage units can be very difficult to effectively clean and disinfect. Ineffective disinfection often leads to an increase in the risk for *Salmonella* contamination due to the supply of additional moisture. The feeding system is also frequently contaminated by rodents which are difficult to control in cage and manure pit systems.

5.8. On-farm storage of feeds

Ideally all ingredients and compound feedingstuffs used on farms should be stored in sealed bulk bins or in bags held in rodent and bird-proof enclosures. Unfortunately this is rarely the case on sheep, cattle and outdoor pig units where feed is often stored in heaps on the floor or in open bins or troughs. Modern chicken breeding, rearing and fattening units normally have enclosed feed bins but slave hoppers may be open and subject to contamination by rodents and birds. Often residual finishing ration left in hoppers is dumped onto the ground and then taken to other farms. Feed fed in dairy milking parlours is often stored in lofts above the parlour where wild birds nest. Ad-lib hoppers often have no lids so are attractive to wild birds, rodents and cats as the upper layers of feed are undisturbed by animals. Farm assurance schemes are concentrating more on secure storage of feed and this has generally improved on large farms included in their schemes, although there may still be issues relating to temporary storage facilities for ingredients prior to drying, or equipment used for other purposes such as manure handling.

In conclusion, it is important that feed ingredients and compound feedingstuffs used on farms are stored and fed in a way which prevents and controls introduction, multiplication or persistence of pathogens in the feed or feeding systems, according to the principles of GHP.

6. Sampling and isolation methods for *Salmonella* in feed.

6.1. Sampling for *Salmonella* in feed

Sampling plans for microorganisms have been designed based on statistical analyses, such as those suggested by ICMSF in 1986 (Legan *et al.*, 2001). A similar approach was used to design sampling plans for *Salmonella* in the Swedish *Salmonella* control program (Ekbohm, 1993). Using these approaches it is possible to calculate the probability of detecting contamination that is confined to, for example, 5% of the lot. In reality the performance of a sampling plan is also determined by the concentration of the organism (Legan *et al.*, 2001). For *Salmonella*, essentially no information is available on the actual concentrations in feed materials or the risk associated with a particular level of contamination.

Mechanical sampling for *Salmonella* is the method most often used as well as automatic sampling. It is generally agreed that a large number of small samples are needed to accurately estimate *Salmonella* in a lot. The heterogeneous distribution of *Salmonella* in feed materials requires sampling procedures that are adapted for this situation. It is well known that dust and fine particles are more likely to be contaminated with *Salmonella*. Thus the sampling of dust in filters or other equipment in the processing line is a good indicator if *Salmonella* is present in the mill. In some countries the *Salmonella* control programme for feed mill is based on scrapings from different parts of the processing line, and not on testing of feed materials.

6.1.1. Design of sampling plans

The uncertainty in sampling is largely dependent on how the contaminant is distributed in the lot. The more unevenly distributed the contaminant is, the more samples are needed to obtain

the same level of confidence. It is recognized that a large number of samples must be taken to increase confidence in negative results (McChesney *et al.*, 1995).

One approach that was applied in sampling plans for microorganisms was to construct a hypothetical distribution based on theoretical assumptions. One might e.g. state that the sampling plan should give a 95% probability of detecting a contaminant that is present in as little as 5% of the lot. This strategy was applied in a control program for *Salmonella* in feed ingredients since many years (Ekbohm, 1993). The limit for *Salmonella* in the EU is "absence in 25g". The method was proposed by (Foster, 1971)saying that "at very low levels of contamination it may be more meaningful to talk in terms of concentration per unit" (reviewed by (Legan *et al.*, 2001). As an example it was given that "an average proportion of 0.05, 0.1 and 0.2 positive 25 g test-units correspond, respectively, to one organism in 500g 250g and 125g of product, respectively". This calculation assumes that *Salmonella* is homogenously distributed in the lot.

Very little information seems to be available in the scientific literature on sampling plans for *Salmonella* or quantitative data on occurrence of *Salmonella* in feed or food. Similar conclusions were made in a recent study by a Norwegian expert panel (Lunestad *et al.*, 2006). In a few older studies where most probable number (MPN) techniques were applied low levels of *Salmonella* were reported (Gunnert and Brest, 1969).

Despite the relatively large numbers of publications reporting figures of the prevalence of *Salmonella* in raw materials and feed there are essentially no data regarding the uncertainty of the sampling procedure something which is discussed in the zoonoses report (EFSA, 2006a, EFSA, 2007c).

A survey of a poultry feed mill (Whyte *et al.*, 2003) frequently detected *Salmonella* in swab samples from transport vehicles and dust samples from the post-heat area, despite negative results in all samples from compound feedingstuffs. The sampling of imported soybeans to Norway (Chapter 4.1.2.2) is based on dust samples from unloading equipment and surrounding environment in the crushing plant. This supports the view that sampling according to HACCP is a more sensitive and cost effective method than traditional sampling of the finished product or feed materials.

6.2. Methods for isolation of *Salmonella* in feed

Growth-based isolation and identification methods using enrichment and selective media are used as the primary means to detect *Salmonella* in the feed chain. The low water activity of most feed materials create an environment where the bacterial cells are strongly dehydrated and thus the isolation method must be able to give injured and stressed cells the possibility to recover and multiply. Cultural as well as immunological methods for *Salmonella* in feed were reviewed by Maciorowski *et al.* (Maciorowski *et al.*, 2006) and the PCR-methods by Maciorowski *et al.* (Maciorowski *et al.*, 2005).

6.2.1. Cultural methods

The international standard cultural method for detection of *Salmonella*, ISO 6579, consists of a non-selective pre-enrichment in Buffered Peptone Water, selective enrichment in Rappaport-Vassiliadis (RVS) and Muller-Kaufmann tetrationate-novobiocin broth (MKTTn) plating on selective solid medium Xylose Lysine Deoxycholat agar (XLD) and another selective medium such as Brillant Green agar (BGA) and finally serological and biochemical confirmation.

Other culture based methods used for *Salmonella* in feed is the NMKL-71 method (Maciorowski *et al.*, 2006). Validation studies of culture methods for *Salmonella* in different feed materials do not seem to have been carried out. A major drawback of the culture methods is the time requirement because most protocols use 5-7 days. Although direct plating may give

rapid preliminary results the low levels of *Salmonella* present in feed require selective enrichment of the samples.

6.2.2. Immunological methods

Immunological methods generally apply enzyme-linked immunoabsorbent assay (ELISA). In ELISA assays, enzyme linked mono- or polyclonal antibodies are used to detect somatic or flagellar antigens from *Salmonella* (Maciorowski *et al.*, 2006). A main advantage is that those methods are more rapid than most cultural methods and also possible to automate. In combination with magnetic beads these techniques can also enhance the isolation of *Salmonella* from large samples. A drawback is that cross reactivity with antigens in related bacteria may cause false positives. The immunological methods may also be less effective for detecting stressed or damaged bacteria.

6.2.3. Molecular methods

Molecular methods for the detection of *Salmonella* include conventional PCR and Real-time PCR. The PCR technique is based on the detection of specific DNA sequences in genetic material, Several commercial kits are available, that are capable of detecting *Salmonella* with high specificity (Maciorowski *et al.*, 2005).

A major obstacle is that *Salmonella* levels in feed are typically low, often less than 1 bacterium/g (D'Aoust and Sewell, 1986). Since the volume of a typical PCR reaction does not exceed 50-100µl, the sample must in some way be enriched before the PCR reaction to meet the requirements. Another problem is that many feed ingredients contain substances that may be inhibitory of the PCR reaction. Thus, direct application of PCR detection will require the development of DNA extraction procedures adapted for feed materials (Maciorowski *et al.*, 2005). The PCR methods presented so far produce only qualitative data.

6.2.4. Quantitative methods

The present methods for isolation of *Salmonella* give qualitative results (absence/presence). Using serial dilutions it is possible to estimate the MPN of bacterial cells in a sample. The traditional MPN method is labour-intensive and not suitable for large scale, however, simplified methods based on microtiter plates are being developed by ISO/TC34/SC9 (http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=36534) that may increase the usefulness of this technique and thus improve the present knowledge about the levels and distribution of *Salmonella* in feed materials. Quantitative data of the levels of bacteria are important for developing improved sampling plans. A more general way of generating quantitative data using PCR is to apply a simplified MPN procedure.

6.3. Conclusion on sampling and isolation methods

Salmonella contamination of feed normally involves small numbers of organisms distributed in a non-uniform way within very large consignments of material. The mechanical sampling using grain spears or grab samples which is normally carried out is often insufficient and automated in-line sampling devices should be used more widely to obtain a more representative sample of ingredients and finished products to increase confidence in negative results. In addition to this, indirect indicators of contamination in the throughput of the mill can be used to assess the likelihood of regular contamination of ingredients or process contamination such as resident *Salmonella* in cooling systems. Similar principles can be applied to oil crushers and feed compounders. Examples of suitable indicator sampling points are: dust from intake auger pits, pooled dust from ingredient bins, dust and cleanings from coolers, dust from crumblers and pellet shakers, pooled dust from finished product bins, dust from outloading gantry. A finding

of contamination in these areas should trigger further more detailed investigation of possible sources of this.

It is not possible to quantify the risk that infectious levels of *Salmonella* would remain undetected with the present sampling plans, however, it is relatively common for *Salmonella* infection of feed origin to be identified in poultry flocks and subsequently traced back to a contamination incident in a feed mill. *Salmonella* surveillance results from animals and timely publication can therefore serve as an efficient alert system for possible feed contamination, particularly if unusual serotypes are involved

Since *Salmonella* present in a feed sample is normally not difficult to isolate it is better to focus on testing more samples with a simple effective method, than fewer samples with a laborious and expensive technique. The ISO6579:2002 (Annex D) MSRV based method has been adopted as the EU standard method for monitoring zoonotic *Salmonella* in samples from primary food animal production. This method also performs well for feed samples but has not yet been formally validated, and it is desirable for this validation to be carried out as soon as possible.

However, the long term use of available methods as a part of a HACCP based control of *Salmonella* in both crushing and feed mills, is found to be a reliable strategy for the production of a feed that does not transmit *Salmonella* even to highly susceptible animals like newly hatched chickens.

7. Assessment of the contribution of feed as a source of *Salmonella* infections in animals and humans.

7.1. Assessment of to what extent feedingstuffs contaminated with *Salmonella* can contribute to the prevalence of infection with *Salmonella* in animals.

The risk of acquiring *Salmonella* from feeding stuffs relates to the frequency of contamination and the dose ingested. Several studies have linked contaminated feed to the occurrence of *Salmonella* in poultry (Durand *et al.*, 1990; Izat and Waldroup, 1990; Primm, 1998), but also in pigs and cattle. Pulsed-field gel electrophoresis restriction patterns of DNA has been used to demonstrate that isolates with indistinguishable fingerprints can be isolated from feed and food producing animals or eggs (Haggblom and Aspan, 1999; Shirota *et al.*, 2001). Contaminated feedingstuffs may not only cause temporally transient animal infections, but may also give rise to the establishment of infections at the farm level that will contribute to infections in subsequent animal batches, and of the farm environment. When most probable number estimates of *Salmonella* levels are carried out on compound feedingstuffs they are usually very low (Taylor and McCoy, 1969) but it is not certain whether it is individual organisms which are being counted or microcolonies intrinsically attached to small feed particles. Furthermore, experimental data on infective doses cannot be applied to contaminated feed without additional considerations. Infective doses for *Salmonella* are normally derived by introduction of broth cultures into experimental animals, and in this state the organisms are highly exposed to intestinal defence mechanisms compared with organisms present in feed which may be protected by fatty material and cause infection with very low numbers (Jones *et al.*, 1982). The infective dose is also lower for animals under stress (such as poultry at the onset of lay), those suffering intercurrent disease, and very young animals where the infective dose can be below 1 cfu/g (Schleifer *et al.*, 1984; Hinton, 1988). *Salmonella* present in low numbers in feed may, in addition, multiply in warm moist conditions such as feed bins which are subject to condensation and ad-lib feed hoppers where feed aggregates moistened by saliva may build-up.

Several studies have shown strong links between contamination of feedstuffs or feed mills and infections of groups of chickens (Boyer *et al.*, 1962; Shapcott, 1985; Wierup *et al.*, 1988; Davies *et al.*, 2001a), turkeys (Zecha *et al.*, 1977; Primm, 1998; Nayak *et al.*, 2003), pigs (Newell *et al.*, 1959; Kranker *et al.*, 2001; Davies *et al.*, 2004; Österberg *et al.*, 2006) and cattle (Glickman *et al.*, 1981; Jones *et al.*, 1982; Davis *et al.*, 2003) with *Salmonella* of the same serotype. Contaminated haylage (Glickman *et al.*, 1981) and vegetable fat (Jones *et al.*, 1982) were shown to be the cause for *S.* Anatum or *S.* Mbandaka infections respectively, in cattle. In addition, risk factor analyses and case control studies have incriminated feedmills in poultry and cattle infections (Anderson *et al.*, 1997; Chadfield *et al.*, 2001). Serotype patterns in animals can suggest feedmill sources (Snow *et al.*, 2007), but most strains found in feedstuffs do not become established on farms (Shapcott, 1985; Veldman *et al.*, 1995); there is likely to be a strong filtering effect exerted by the endemic farm microflora, animal susceptibilities, feed storage conditions and doubtless many other factors.

Examination of surveillance data indicates that feedstuff *Salmonella* overlap to varying degrees with clinical or surveillance-derived serotype from animals. In the UK in 2005, the commonest serotypes isolated from poultry feed (*S.* Livingstone and *S.* Kedougou) were also among the top three isolated from chickens (*S.* Livingstone, *S.* Senftenberg and *S.* Kedougou in rank order). In the same report (Anon., 2006b), *S.* Typhimurium was the only serotype of the relatively few feed isolates to coincide with the top three serotypes reported from cattle, sheep and pigs, respectively. The data from the EU as a whole (EFSA, 2006a; EFSA, 2007a, b, c) shows more regional variation, as would be expected, and suffers from variability in methodologies and which member states contribute to each data class. Notably, however, *S.* Typhimurium is prominent among isolates from chickens, pigs, ruminants and additionally from feedstuffs. Serotypes Infantis and Mbandaka are common amongst broilers, layers and feedstuffs, but conversely it should also be noted that the top three feedstuffs serotypes (*S.* Livingstone, *S.* Senftenberg and *S.* Montevideo) are not apparently widespread and common elsewhere, whilst serotypes Enteritidis and Hadar are common amongst layers and broilers but not feedstuffs.

Consideration of individual studies clearly shows the potential of feedstuffs to infect groups of animals with *Salmonellae*, but surveillance data is more equivocal on the matter of its relative importance. Certainly there is overlap between the serotypes commonly found in feed and in livestock, with *S.* Typhimurium being a case in point. Although persistent environmental contamination has been shown to be a major factor in the infection of layer flocks (van de Giessen *et al.*, 1994; Davies and Breslin, 2003; Gradel *et al.*, 2004), contaminated feedstuffs may make a significant contribution to the problem, particularly as layer rations typically are not heat-treated, and may in particular be an important route for the infection of previously uncontaminated henhouses. Similarly, endemic infections on pig premises are likely to be of primary importance, but contaminated or recontaminated feed is considered a significant risk factor and may account for 15-30% of *Salmonella* infections in the finishing period (Berends, 1996). Feed contamination will be of increased importance on units or regions with low prevalence status where endemic infection is well-controlled or absent (Shapcott, 1985), For example in Sweden feed is the major source when *Salmonella* is found to be introduced in particular to swine and poultry meat production (Wierup, 2006). *Salmonella* infection with currently-prevalent serotypes is rarely seen to cause health problems among chickens, but enteric and other clinical disease is seen more often with turkeys and pigs, and probably most frequently amongst infected ruminants. Young weaning pigs are commonly affected by *Salmonella* infection in the feed in countries where routine medication is not used, and in some cases, the infection causes clinical disease (Sauli *et al.*, 2005). Adult pigs have also been shown to be susceptible to infection (van der Wolf *et al.*, 1999; Österberg *et al.*, 2006). Another study (Berends, 1996) estimated that about 15-30% of all infections in the finishing period may be attributed to contaminated feed. Data from studies on dairy farms suggests that subclinical infection in cattle carried by *Salmonella* serotypes associated with feed is common (Davison *et*

al., 2006) and some of these strains may subsequently be involved in human illness (USDA:APHIS:VS, 2005). Where there is a major source of endemic *Salmonella* infection, such as animal to animal spread (e.g. on pig farms or calf units) or persistent environmental contamination (e.g. commercial layer farms) feed may be a relatively less prominent source of infection. In cases such as all-in/all-out production of broiler and turkey breeding, and production flocks which are operated to a high standard of biosecurity, feed becomes a relatively more important source.

7.2. Assessment of to what extent feedingstuffs contaminated with *Salmonella* can contribute to the contamination of food produced from animals.

Food is the major route of transmission of non-typhoidal *Salmonellae* to humans (Mead *et al.*, 1999; Crump *et al.*, 2002), and animal food products (meat, eggs and dairy) are the vehicles primarily implicated (Anon., 2006a; EFSA, 2006a; EFSA, 2007c). Eggs and chicken products are particularly strongly represented in recent data (Anon., 2005), and confirmed foodborne outbreaks of human salmonellosis in the EU show a heavy predominance of *S.* Enteritidis, with *S.* Typhimurium being the second in rank. The same report also ranks Enteritidis first amongst serotypes isolated from eggs and broiler meat, whilst Typhimurium predominates in isolates from pig meat, and is also prominent in isolates from beef and chicken. It has been demonstrated that *Salmonella* strains, including *S.* Typhimurium, from broiler feed sources can correlate with those found in birds and on derived broiler meat (Pennington *et al.*, 1968; Semple *et al.*, 1968; MacKenzie and Bains, 1976; Davies *et al.*, 2001a,b; Corry *et al.*, 2002). In a Danish risk analysis, pork and beef were proposed as vehicles for *Salmonella* originating in pig and cattle feedstuffs, accounting for approximately 2% of human *Salmonella* cases (Hald *et al.*, 2006). In another study (Newell *et al.*, 1959) uncovered evidence of links between *Salmonella* contamination of pig feedstuffs, slaughter pigs and pork products.

Egg contamination in the EU, typically by *S.* Enteritidis, is not likely to be greatly influenced in the short term by feedstuffs, as these are uncommonly contaminated by *S.* Enteritidis. However, in Japan, correlations between both the degree of contamination and the strains of *S.* Enteritidis present in feedstuffs and eggs have been demonstrated (Shirota *et al.*, 2000; Shirota *et al.*, 2001) and feed is postulated as a possible initial source of epidemic *S.* Enteritidis before trade in infected breeding stock became the predominant route (Evans *et al.*, 1999). More recently a link between pelleted broiler feed contaminated with various *Salmonella* serotypes, including a particular phage type/genotype of *S.* Enteritidis, and raw chicken nuggets and strips has been reported from Canada (Bucher *et al.*, 2007). This possible link between feed and food and subsequent human exposure of serotypes commonly pathogenic to humans can also be considered in the EU where, as can be seen from the EFSA zoonoses report (EFSA, 2006a; EFSA, 2007c), S. Enteritidis as well as *S.* Typhimurium occasionally were occasionally isolated from feed. Milk and other dairy products are another potential route for *Salmonella* infection of humans, and evidence for such a route from feedstuffs to dairy cow to milk has been presented (Knox *et al.*, 1963).

As the link between feed contamination by *Salmonella* and infection of animals has been established, and the level of *Salmonella* contamination of animals arriving at abattoirs can affect the level of contamination of carcases leaving the plant (Campbell *et al.*, 1982), then it is logical to suppose that contamination in feedstuffs can affect contamination in meats, and this has been shown in some cases. The potential route via eggs or milk is even more direct. However, the chain of transfer is unlikely to be uniform or straightforward. If the food product (for example, eggs) is not usually contaminated with a serotype that reflects the feed strains, then the effect of feed contamination will be minor, at least in the short- to medium-term. If the food product is effectively heat treated (for example, milk), then the long-term risk from contamination, even if a food-related outbreak amongst the milking animals is undiagnosed, is

likely to remain low for the public, if not for the farm workers. The passage of *Salmonella* through an abattoir may be considerably reduced by hygiene and decontamination processes, and there exists the possibility that a *Salmonella* strain in incoming animals is supplanted or joined by a previously-introduced strain in the plant.

It would appear that the risk of feedstuff-acquired *Salmonellae* appearing in human food is greatest for those livestock species where unapparent infection is usual (i.e. chickens, pigs and turkeys) and which commonly maintain serotypes that are seen regularly in feedstuffs and are established to be of high virulence in humans, the prime example being *S.* Typhimurium.

7.3. Assessment of to what extent feedingstuffs contaminated with *Salmonella* can contribute to the prevalence of *Salmonella* cases in humans.

The overlap between *Salmonella* serotypes commonly found in animal feedstuffs and those isolated from human cases of salmonellosis is limited, but across the EU, four of the serotypes ranked in the top ten feed isolates (Infantis, Typhimurium, Agona and Enteritidis) are also in the top ten public health serotypes (EFSA, 2006a). This at least suggests the potential for feedstuff strains to pass far enough up the food chain to cause human disease. *S.* Hadar was found in Britain in poultry offal meal imported from Israel in 1969, and it became endemic in turkey breeding flocks in 1973-74 (Watson and Kirby, 1985). Within a few years of this it had moved from a very rare to a very frequent human isolate in the UK, and a route to humans via turkey products was established (Rowe *et al.*, 1980).

There have been a number of historical reports which establish with some confidence a direct link from human salmonellosis cases through animal products to animal feedstuffs. Bone meal contaminated with *S.* Hadar and fed to chickens was linked to *S.* Hadar infections contracted from eating the chicken livers (Hirsch and Sapiro-Hirsch, 1958). Meat and bone meal contaminated with *S.* Heidelberg was implicated in a milkborne outbreak of the same serotype (Knox *et al.*, 1963). In 1968, linked papers (Pennington *et al.*, 1968; Semple *et al.*, 1968) reported that an outbreak of *S.* Virchow in humans was traced back to a poultry enterprise, where there was contamination of both the hatchery and the food. A primary or secondary role for feed contamination was postulated. Possibly of most significance is the reported novel appearance of *S.* Agona in the USA, the UK, the Netherlands and Israel in 1969-70 (Clark *et al.*, 1973). Investigations in each country established a chronological sequence of isolations from peruvian fishmeal, livestock and then humans, and a detailed study in the southern USA traced human infection back via chickens to imported Peruvian fishmeal. This case has particular impact because of the subsequent sustained level of poultry and human infections (> 1 million cases) with this serotype over the subsequent two decades (Crump *et al.*, 2002). A similar situation is currently occurring with *S.* Agona, which regularly contaminates vegetable proteins and is found in infections in turkeys. *S.* Rissen is another *Salmonella* serotype which appears to have passed from vegetable proteins to turkeys in recent years, but is uncommon in humans (Anon., 2006b).

In a Danish study (Hald *et al.*, 2006) it was estimated that up to 2.1% of the domestically acquired human salmonellosis cases in the period 1999-2003 could be attributed to feed-borne serotypes.

Despite such evidence, differences in serotypes isolated from humans and from feedingstuffs are sometimes used as an argument to claim that feed does not contribute substantially to human food-borne illness (Crump *et al.*, 2002). The issue has been discussed intensively, and several aspects have to be considered such as the efficiency of sampling of feed-producing facilities and the 'filtering' effect which relates to the infectivity and pathogenicity of different serotypes in different hosts (chapter 4.1.4). It is also likely that feed may have been involved in the international dissemination of 'epidemic' strains of *Salmonella* such as *S.* Typhimurium DT104 (Davies, 2001b; Helms *et al.*, 2005).

7.4. Ranking the risk posed by feed as a contribution to *Salmonella* infections in animals and humans in comparison to other possible sources.

Although opinions vary on the importance of feedstuffs contamination (Jones *et al.*, 2004a) there is a substantial body of evidence that in many situations feedstuffs can pose a significant risk of *Salmonella* infection for humans and animals. Although there is limited overlap between common human and feed serotypes, the aggregation and ranking of data on serotypes may obscure regional patterns. For example, serotypes Enteritidis and Typhimurium appear to be uncommon in feed in many reports (Bisping, 1993), but more common elsewhere (MacKenzie and Bains, 1976). Some authors consider feed to rank alongside imported pigs for the risk of *Salmonella* introduction (Sauli *et al.*, 2005), and the link between feedmills and *Salmonella* in pigs is well-established (above), whilst on-farm mixing of feed may also be a risk factor for introduction of *Salmonella* in pig herds despite being partially protective in terms of the risk of a high within-herd prevalence of endemic *Salmonella* (Davies *et al.*, 2004). Feed is cited by some authors as a major source for cattle herd infections (Eddy, 2004; Jones *et al.*, 2004).

In broiler production, both hatchery and feed contamination are implicated in *Salmonella* strains seen at slaughter (Corry *et al.*, 2002), and among UK layer flocks the use of certain feed mills is a risk factor for *Salmonella*, including *S.* Enteritidis (Snow *et al.*, 2007). This last point is unexpected, as *S.* Enteritidis is currently uncommonly found in UK feedstuffs. Feed contamination, including the use of poultry offal and feather meal as well as indirect contamination of other feed materials from environmental sources, is likely to have played a greater role in the early stages of the *S.* Enteritidis epidemic in UK poultry. However, feed sampling may only reveal part of the picture, as sample size has a substantial effect on *Salmonella* recovery (Shirota *et al.*, 2000), and there is also the filtering effect of farm and animal environment to consider, potentially resulting in certain occasional contaminants being ultimately more successful colonisers than some of the more common isolates.

The effects of feed contamination should also be considered on short-term and long-term timescales. With short-cycle production such as broilers, feed contamination can be seen to introduce new serotypes and to fairly quickly influence the serotypes seen at slaughter and on carcases (MacKenzie and Bains, 1976). The effects on longer-term production cycles that may have endemic *Salmonella* strains, such as in layers and pigs, may be more subtle but ultimately still significant, particularly when efforts to reduce the level of infection are being made in a unit or if the feed-related *Salmonella* gains access to the higher levels of pig breeding organisations and integrations.

In conclusion, in the same way as *Salmonella*-contaminated food is the major source of *Salmonella* infections in humans, animals face a similar risk of becoming infected when fed with *Salmonella*-contaminated feed. However, animals may also be perorally infected from faecal contamination from other infected animals or via a faecally contaminated environment for which the primary source is also likely to be *Salmonella*- contaminated feed. Contamination of feed ingredients during production, storage, transport and processing by *Salmonella* serotypes originating from the faeces of wild or domestic animals, contaminated water or processing equipment is common. Animals such as poultry and pigs which derive all or the greatest part of their nutritional requirements from compound feed are most at risk but the relative importance of feed as a source depends on the coexistence of other sources of infection. Such predominant sources include movements of infected animals, infected wildlife vectors or residual environmental contamination, for which the primary source may be *Salmonella* contaminated feed. The importance of *Salmonella* infection from feed varies according to the position in the breeding and production pyramid where infection occurs. For example, if infection occurs in a primary pig or poultry company it may be distributed worldwide by international trade in breeding stock, as well as resulting in perpetual infection on continuously occupied primary and commercial breeding units which then becomes an ongoing source of

Salmonella for an indefinite period. Occasional introductions of *Salmonella* into parent breeding or commercial broiler and turkey flocks or fattening pig herds which are operated on an all-in/all-out basis present a shorter-term risk but may persist if cleaning and disinfection is not effective or may contaminate hatchery or abattoir equipment, which may occasionally also be a long-term problem.

8. Strategies to control *Salmonella* in the feed-chain

8.1. HACCP principles, Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP) systems

Regulation (EC) No 183/2005 of the European Parliament (http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_035/l_03520050208en00010022.pdf), laying down requirements for feed hygiene, indicates that "Feed business operators shall put in place, implement and maintain, a permanent written procedure or procedures based on the HACCP principles".

The feed production industry has a relevant role in the food chain. In order to ensure production of safe feed, HACCP principles, and GHP/GMP systems should be applied at each stage of the feed chain, from feed materials, to feed processing.

The European Feed Manufacturers Guide (http://www.fefac.org/code.aspx?EntryID=265, FEFAC) and The Feed Ingredients Standard (http://www.ifsa-info.net/lmbinaries/ifis.pdf, IFSA) in accordance with Article 22 of Regulation (EC) No 183/2005 were published in 2005 and 2007 respectively as a guide to good practice for the industrial EU compound feed and premixtures manufacturing sector for food producing animals.

In the UK there are numerous voluntary codes of practice concerning the various stages of production of feed and the UK situation is summarised in a Government report (HMSO, 1992). New revisions of the codes of practice are currently being formulated.

8.1.1. Primary production of feed

At the level of the primary production of feed, contamination with *Salmonella* spp. is possible through the spreading of contaminated fertilizers (slurry, manure, waste sludge…) on the pasture / fields, ingredients and co-products.

The risk of contamination of the fertilizers with *Salmonella* spp. can be decreased by implementing of several procedures such as:

- Storage of the fertilizer for more than 2 months, without any new influx,
- Composting,
- Ploughing in after spreading fertiliser,
- Increasing the time allowed between spreading of the fertilizer and the animal grazing or crop harvesting,
- Heat treatment of the fertilizers before use,
- Treating fertilisers with the addition of lime,

Animal feed ingredients can be also contaminated by *Salmonella* spp. during processing because of a residual contamination of the premises, equipment and staff. This risk of contamination can be increased because of an insufficient cleaning and disinfection of the premises and equipment used to process the feed crops, but also because of poor hygiene conditions during storage, poor hygiene of the staff, or environmental conditions (moisture and

temperature) favourable for *Salmonella* multiplication. *Salmonella* can also be spread through operators, vermin, cross contamination, recontamination after processing, etc.

The risk of contamination can be decreased by procedures including:

- Heat treatment on the co-products during processing,
- Cleaning and disinfection of the facilities, and control its efficiency,
- Implementation of good hygiene practices,
- Controlling the moisture of the co-products,
- To carry out routine bacteriological controls on the vegetables and the resulting co-products,
- Cleaning the silo and other storage equipment,
- Maintaining good ventilation of the silo,
- Controlling vermin during storage and transport.

These existing procedures could be improved by selection of the suppliers based on their implementation of programmes for the control of *Salmonella*, systematic control of the batches at delivery, and to systematically auditing of the suppliers.

There is also monitoring performed by the processors themselves, consisting in sampling and testing during processing, control of the end-products (co-products in that case), plan of survey, control of the flows, temperature, hygiene, cooking, packing, validation of the co-products shelf life.

But even if there are solutions to limit the *Salmonella* contaminations, the control of vegetable products is performed *a posteriori*, and the response to give in case of contamination is not clearly defined. Moreover, methods of *Salmonella* quantification are lacking.

8.1.2. Transport and storage of ingredients

The importance of conditions of transport and storage of the ingredients has been already addressed. The risk is increased by poor hygiene and no respect of good practices, mainly poor cleaning and disinfection of containers, wheels, equipment for collection, and silos, and also by the presence of vermin and wild birds.

The procedures to decrease the risk of contamination may follow these recommendations:

- All means of transport (whether by ship, barge, road vehicle, rail, container or other transport system) of ingredients as well as compound feedingstuffs must be made by using only hygienic vehicles and in compliance with a transport guide or relevant transport sections of sectoral guides developed in accordance with article 22 of Regulation (EC) No 183/2005.
- All means of transport whatever are the situation (owned or contracted, in bulk or packed) must be adequately controlled with specific regard to hygiene and potential contamination. Cleaning and disinfection of the trucks between each batch.
- All vehicles used for delivery must be kept clean and operated according to a transport Guide: The transport Guide must prescribe that all vehicles used for the transport of incoming and compound feedingstuffs must be subject to regular cleaning and sanitising programmes ensuring that these are in a clean state, with no accumulation of residual waste material.

- Incoming and compound feedingstuffs must be protected from contamination and kept dry during transport. Enclosed vehicles or containers must be used whenever possible for loose bulk, but where this is impracticable, the loads must be covered. The cover used must be maintained in a clean condition by being cleaned, sanitised and dried regularly.
- No materials from previous loading must remain in the container (tank truck, boxes). The container must be clean and dry.
- Incoming and compound feedingstuffs must be protected from contamination and kept dry during transport. Enclosed vehicles or containers must be used whenever possible for loose bulk, but where this is impracticable, the loads must be covered. The cover used must be maintained in a clean condition by being cleaned, sanitised and dried regularly.
- In the absence of transport Guides for compound feedingstuffs, other proofs of hygiene and traceability of previous loads must be specified.
- Cleaning and disinfection of the silos
- Controlling vermin contamination during transport and storage
- Avoiding access of wild birds to the storage facilities.

Many professionals follow internal guide to promote good hygiene in transport and storage.

However, the response to give in case of contamination is not clearly defined and methods of *Salmonella* quantification are lacking.

8.1.3. Feed mill

8.1.3.1. Handling and storage of ingredients

The contamination risks present at this stage of feed production are mainly if the ingredients are contaminated with *Salmonella* upon arrival at the feed mill or the feed mill environment carries a permanent *Salmonella* infection that will contaminate the ingredient. As was mentioned earlier in this report it is not unusual that ingredients of plant or animal origin carry *Salmonella* infection which may be associated with contamination at production or contamination during transport (Davies and Wray, 1996; Crump *et al.*, 2002; Jones and Richardson, 2004).

Experience has shown that a feed mill that received a contaminated ingredient may become contaminated for an extended period of time. Unloading ingredients in the intake pit creates large amounts of dust which may carry *Salmonella* infection to the premises. The transport equipment used for ingredients may become contaminated as well as the flat storage areas and silos due to contaminated dust particles remaining inside the systems. If intake pits or other parts of the transport or storage systems carry infection since the previous ingredient *Salmonella* negative ingredients may also become contaminated.

Intake pits easily attract vermin and wild birds and automatic doors surrounding the pit offer some protection to contamination from faecal material from wild animals. An efficient dust control in the pit area is very important to prevent further spread of *Salmonella* from potentially contaminated ingredients.

Water from trucks or rail cars entering the intake pit due to rainfall or leakage through roofs of storage buildings is not unusual and can lead to increased moisture content in the commodity giving rise to actual multiplication of *Salmonella* in ”hot spots” of the ingredient. In feed mills situated close to the sea the lower end of elevators are usually below see level, a situation

which temporarily might generate moisture levels in the dust above the level where *Salmonella* multiplication may occur.

Ingredients entering the feed mill with elevated temperatures or when warm days are followed by cold nights may cause condensation of free water on cold surfaces in the transportation systems and also in storage containers. A study (Carlson and Snoeyenbos, 1970) pointed out the possibility of condensation in ingredient storage containers as an environment capable of supporting growth of *Salmonella*. Ventilated silos will reduce the build up of moisture and free water in the top of the silos and prevent the possible multiplication of *Salmonella*.

Flat stores are attractive to wild birds and rodents and for that reason effective control measures are important to prevent contamination.

It is important that silos are cleaned regularly as well as flat storage areas. A cleaning programme for the ingredients will reduce the potential build up of *Salmonella* in the premises and insulation of storage containers will reduce the potential build up of condensation.

8.1.3.2. Design of the feed mill

It is important to physically separate all possible contacts between ingredients and compound feedingstuffs, however it is not unusual that there is no clear separation within the feed mill especially in older premises.

Ingredients must not be stored in bins or silos for finished products or the opposite. Air with contaminated dust moving from ingredients to the processed feed e.g. in the cooler is a possible source of re-infection of the feed. Any portable equipment used in the feed mill is a potential source of *Salmonella* re-infection of the heat treated feed. Repair work of closely situated conveyers or elevators used for ingredients or finished products is another potential source of infection for the heat treated products.

8.1.3.3. Processing

Bins, blenders, mixers and conveyers should be constructed in such a way that the build up of materials within the equipment is minimized and should also provide access to inspection and cleaning. Grinding and mixing is not considered to increase the total bacterial numbers in the mash feed (Hall and Tallentine, 1978). No major risks for multiplication of *Salmonella* seem to be present in this part of feed manufacturing (Davies and Hinton, 2000). Any equipment with limited and labours access tends to be inspected and cleaned less often than equipment with an easy access. Any leakages, spillages or dust accumulated on floors or elsewhere in the premises may cause dissemination of *Salmonella* in the mill particularly if the leakages occur from the ingredients in areas of the feed mill where the finished product is exposed to the environment.

8.1.3.4. Conditioning and pelleting

Conditioning, followed by pelleting or expanding are normally the only processing procedures performed by a feed manufacturer that can completely destroy *Salmonella* in the mash. Typically, the meal is introduced into the conditioner where steam is added to raise the temperature to the preset temperature. The temperature and time used during conditioning will primarily determine the antibacterial effect of the process, while the friction generated at the actual pelleting, when the meal is passed through the die of the pellet press, will raise the temperature instantly by a few degrees. Pelleting usually involves temperatures between 70 and 90 C and with the times used in commercial feed production an expected reduction of 99% (2 logarithmic reduction of the total bacteria) will occur (Hall and Tallentine, 1978; Furuta *et al.*, 1980; McCapes *et al.*, 1989). Temperatures of 80^{o}C in the conditioner followed by pelleting will in most cases be sufficient to kill the *Salmonella* bacteria present (Blankenship *et al.*, 1984), except when there is an extremely high rate of bacterial contamination in the ingredients

or if the feed is conditioned and pelleted at lower temperatures and time. Extended steam conditioning up to 30 seconds or more have been shown to increase the antibacterial effect of the heat treatment. For these reasons it is therefore important that pellet mills are carefully monitored to insure proper operation with respect to temperature and retention time in the conditioner particularly the period after the production started when optimum antibacterial conditions do not prevail.

Steam going into the conditioner may accumulate on the floor around the pellet press due leakage and the conditions might become ideal for bacterial multiplication as it is not unusual that wet meal accumulates under the pellet press. Cracks in the floor where *Salmonella* bacteria are present in the dust or underneath damaged paint is difficult to disinfect and may harbour *Salmonella* spp. for long periods of time.

Condensation will occur if the temperature difference is more than 5^{o}C between the pelleted feed and the environment. It is not unusual that coolers are not efficient enough to produce a low temperature of the pellets thus the warm pellets will give rise to condensation and free water in "clean side" of the feed mill. Condensation usually occurs as droplets of water and if the conditions favour growth, a single droplet typically in the top of the conveyer or silo, may contain large numbers of bacteria. Insulation of the top of the cooler will reduce the risks of condensation.

Because of the very large amounts of cooling air used in the coolers the microbial quality of the air is very important. The source of cooling air is critical and should not originate from ingredient receiving sites or loading areas (Jones and Ricke, 1994). Contaminated dust particles in the air will be picked up by the feed in the cooler and potentially contaminate the cooler especially if condensation occurs in the top.

Salmonella bacteria will occur on the roof of the feed mill if the cooler is infected by *Salmonella*. Air from outside the feed mill should not be taken close to outlets from the cooling process. Air going into the coolers from the mill might sometimes be transported also from ingredients if the design of the mill is not well planned. The use of particle filters absorbing most of the dust particles is a measure to secure the quality of the cooling air.

It is not unusual that condensation occurs inside the top of pellets coolers or in the outlet air system, conditions that are favourable for growth because of access to free water and temperatures around the optimum for growth for *Salmonella*. Depending on the temperature of the pellets after the cooling process and the ambient temperature condensation may occur in conveyers or elevators in the feed mill, in the feed truck or even in the storage bin at the farm. It sometimes occurs that conveyers for compound feedingstuffs are situated outside the feed mill a situation which might favour condensation and growth of *Salmonella*.

Other risks for recontamination may be a general aspiration system for ingredients and heat treated products. The storage bins for the aspiration dust may harbour *Salmonella* for extended periods of time. Dust from the aspiration may only be reintroduced in the process before the heat treatment.

A discontinuous production give rise to fluctuating temperature in the processing equipment with the chances that incompletely heat treated feed will go into the cooler or the development of suitable temperatures for microbial growth in equipment with moist feed for extended periods of time.

Separate conveyers and containers for mash and pelleted feed are important to prevent cross contamination and conveyers and containers for compound feedingstuffs should not be used for ingredients. It is unusual that the outloading gantries in a feed mill are strictly separated between mash and pelleted feed a situation which present a certain risk for cross contamination.

8.1.3.5. Decontamination

Thorough physical cleaning followed by efficient chemical disinfection is the most important factor to eliminate persistent *Salmonella* contamination in a feed mill. Dry cleaning using vacuum cleaners are used in most feed mills and the accumulated dust must be discarded. Water should be avoided in the cleaning process because residual water may enter processing equipment or containers and accentuate the *Salmonella* problems. Ineffective disinfection may aggravate the *Salmonella* situation and only the recommended concentration of the disinfectant should be used.

8.1.3.6. Foot traffic

Contaminated dust in the premises can easily be further distributed inside the mill by foot traffic. Sensitive environments around the pelleting and the cooler or the storage bins for compound feedingstuffs may be contaminated by *Salmonella* carried on shoes or clothing. A physical separation preventing foot traffic in these areas will decrease the risks for further spread of contaminated dust. A high degree of cleanliness of clothing and shoes is important to implement among the employees. Special attention should also be given to the involvement of internal and external technical staff and their equipment and tools in the management routines that are to be in place according to the feed hygiene directive 183/2005 (Chapter 9.4)

8.1.3.7. Wild birds, insects and rodents

A feed mill should not be situated close to other industrial or agricultural activities where there is a risk for contamination of the mill environment, wild birds and other wildlife. Wild birds are usually a problem in the feed mill environment and may contaminate the feed by their faeces. Wild birds are most often found in the outloading facilities and can to some extent be prevented by automatic doors for the trucks. Sea birds are usually found in the environment when the feed mill is situated in a coastal area. Sea gulls are reported to be infected by *Salmonella* in certain regions (Duarte *et al.*, 2002). It is important to control rodents by conventional control measures.

8.1.4. Transport and storage on the farm

The main sources of contamination by *Salmonella* during the storage of the feed at the farm are the vermin and the wild birds, but also the presence of carnivorous pets and other animal species on the farm. In addition to the utilisation of contaminated materials and cross contamination with previous batches of contaminated feed or other products stored at the same place. The risk is increased by the strong presence of vermin and wild birds, poor hygiene in silos, the utilisation of the fodder equipment for handling animal excreta for instance, the mixing of contaminated feeds with uncontaminated feeds, etc.

The existing procedures to decrease the risk of contamination by *Salmonella* are:

- To control the moisture of the feed during its storage.
- To proof the basement, cover the silo in order to prevent contamination by wild birds, control vermin, and, maintain a good hygiene during storage and maintain the approaches of the building,
- To limit the number of visitors, respect the hygiene rules (farmer, visitors) and utilise special clothes
- To utilise a unique equipment for the farm and to train the staff for hygiene

The farmer should in addition perform regular cleaning of the silos.

Farmers often perform monitoring by visual checking of the silos and also by the assessment of the efficiency of the plan of campaign against rodents.

8.1.5. Feeding systems

Contamination of the feed during its distribution to animals is also possible, via inert carriage (water, building, equipment, conveyor belts, troughs, packing device, soil, animal excreta…), asymptomatic carriage by animals (vermin, birds, carnivorous pets) and asymptomatic carriage by human staff. The risk is increased in case of insufficient cleaning / disinfecting of the building and equipment, poor hygiene in troughs, the presence of vermin, and the contamination of the drinking water (rare), bad adjustment of the feeders, etc. Moreover, the danger can be spread by animals, vermin, farmer, visitor, lending of the equipment, etc.

Procedures additional to chapter above to decrease this risk include:

- To control the drinking water,
- To clean the building and the feeder's equipment,
- To respect a crawl space,
- To manage effluents.

In addition, possible procedures could be applied such as the utilisation of specialized staff and equipment, a double disinfecting (in case of risked situation).

8.2. Processing conditions aimed at reducing Salmonella contamination

The ingredient or compound feedingstuffs milling process, when run according to the principles of GHP (FEFAC, 1999), has several stages during which bacterial contamination may be reduced (Hess *et al.*, 1970; Jones and Richardson, 2004), but also many opportunities where cross-contamination may occur, especially in vegetable oil/oilseed manufacturing plants (Morita *et al.*, 2006). Storage of ingredients leads to a gradual die off of organisms so newly harvested, dried and processed ingredients normally carry a greater risk than materials which have been stored for a period (Davies and Hinton, 2000) but many ingredients such as oilseed meals are supplied direct from a processor in which case they may be highly contaminated on arrival at the mill unless terminally treated (Nape and Murphy, 1971). Passage of the feed ingredients through auger systems, grinders, sieves and mixers produces frictional, pressure and shear forces (Cooke, 1992; Cooke, 2002) which may have a slight antibacterial effect. These activities may also break up some micro-colonies attached to feed particles so that isolated organisms are less likely to survive. In addition, much of the contamination of feed ingredients is on the surface of solids such as grains, so the handling and cleaning process may remove some of the contamination. All of these processes however are likely to produce only a marginal reduction in bacterial contamination so it is common to introduce a treatment stage.

8.2.1. Heat treatment of feedingstuffs

The most common feed treatment is the use of heat. This is often done as part of the pelleting process to produce a good quality pellet rather than to heat treat the ration. This is particularly true of large pellets or cobs produced for adult ruminants or pigs. Heat treatment systems used in feed mills can be classified as follows (Buick, 2000):

- Short-term conditioners: used for pre-cubing, expanding and extruding - the most commonly used option.
- Long-term conditioners / Ripeners / Kettles: used for pre-cubing.
- Cooker conditioners: used for pre-cubing and pre-rolling.

- Extruders: used for straight raw materials and finished compounds.
- Expanders: used for pre-cubing and production of expandate / crumbles.
- Combination conditioners: used for pre-cubing.
- Heat exploders: used for whole materials.
- Post-pelleting heat treatment: used after pelleting and expanding to retain heat for longer.
- Direct gas/vapour conditioners: used for mashes and pre-cubing.

Rations for rearing poultry, breeding poultry and fattening poultry are commonly pelleted. The first stage rations of these are usually crumbled to encourage uptake. Pig rearing and fattening rations are also often pelleted since fine grinding and extrusion or pelleting may, depending on ingredients (Sun *et al.*, 2006), increase the feed intake, growth rate and feed conversion efficiency of the animals (Vanderwal, 1979). Starter rations for commercial layers are also normally pelleted and crumbed but older rearing and laying birds are normally fed on untreated meal rations. Fine grinding and pelleting may however lead to other problems such as gastric ulceration in pigs and dysbacteriosis leading to necrotic enteritis and 'wet litter' in poultry.

Heat treatment has traditionally been considered the simplest and most cost effective method of decontaminating feed (Saulmon, 1966; Nape and Murphy, 1971; Williams, 1981). Many studies have demonstrated the potential of heat to reduce microbial contamination (Mossel *et al.*, 1967; Stott *et al.*, 1975; Jones *et al.*, 1991; Veldman *et al.*, 1995). The effectiveness of heat treatment is considerably influenced by the constituents of the feed (especially fats) (Doyle and Mazzotta, 2000; Juneja and Eblen, 2000), available water levels (Liu *et al.*, 1969; Farkas, 2001), level and homogeneity of contamination, the temperature profile achieved through the batch of feed and individual feed particles and the minimum treatment period (Ricke, 2005). The efficiency of treatments may also be over-estimated in commercial conditions by ineffective sampling and testing (Williams, 1981) and the long tail of surviving low numbers of organisms which may be difficult to detect (Doyle and Mazzotta, 2000). Factors such as cost, especially with rapidly rising energy costs, heat damage to vitamins and other nutrients and adverse effects on the integrity of the pellets (the appearance of pellets and absence of crumbling is an important marketing consideration) plus the need to maintain a high throughput tends to restrict the period of heat treatment and the maximum temperature to a few seconds (Peisker, 2006).

After grinding and mixing, feed to be pelleted is conditioned or expanded, then pelleted. Heat treatment offers an opportunity to eliminate *Salmonella* and other bacteria from feed ingredients (Ekperigin *et al.*, 1990; Ekperigin *et al.*, 1991) to achieve a *Salmonella*-free finished product, but in practice some *Salmonella* do survive the pelleting process due to deficiencies in the heat-treatment application (Hacking *et al.*, 1978; Cox *et al.*, 1983; Blank *et al.*, 1996). Pelleting also increases feed utilisation efficacy (Jones *et al.*, 1995) and reduces waste during feeding (Vanderwal, 1979; Andrews, 1991). There is little current information on the effectiveness of heat treatment in modern large-scale feedmills (Voeten and van de Leest, 1989). Most studies on the effect of heat treatment have been carried out using artificially contaminated samples but more heat is required to eliminate *Salmonella* from intrinsically contaminated materials which have been contaminated by natural means (Williams, 1981). Studies are subject to considerable variability in methodology, especially when selective culture is used for stressed organisms, so comparison between studies must be carried out with caution (Kobayashi *et al.*, 2005; Wesche *et al.*, 2005). Conditioning and pelleting at 93^{o} C for 90 seconds at 15% moisture has been shown to reduce *Salmonella* by 10,000-fold, which should eliminate all but extremely high contamination levels (Himathongkham *et al.*, 1996) but in practice such high temperatures and conditioning times are rarely used because of the cost of

heat energy and the deleterious effect of increasing moisture associated with very high temperatures on pellet quality (McCapes *et al.*, 1989; Blank *et al.*, 1996; Vest, 1996; Maciorowski *et al.*, 2004) or levels of micro-nutrients (Jones *et al.*, 1995). Pellets were however heated to a minimum of 70° C for 12 minutes as part of a National Control Programme to eradicate *S.* Enteritidis in broiler breeding and production in Northern Ireland (McIlroy *et al.*, 1989). Temperatures of at least 80° C during conditioning followed by pelleting are likely to be successful in the majority of cases (Blankenship *et al.*, 1984; Coven *et al.*, 1985; Veldman *et al.*, 1995) as *Salmonella* most-probable-number estimates in feed are usually less than 1/g (Taylor and McCoy, 1969). 80-85° C for 30 minutes eliminated all enterobacteriaceae from feed meal (Kampelmacher *et al.*, 1965). 85° C for one minute is considered a good target requirement for *Salmonella* elimination (Mossel *et al.*, 1967; Liu *et al.*, 1969; Jones and Richardson, 2004) but in practice conditioning temperatures are normally lower and times are usually much shorter than this, so some contamination is likely to survive in many cases (Stott *et al.*, 1975; Cox *et al.*, 1983; Jones *et al.*, 1991; Davies, 1992; Veldman *et al.*, 1995). Samples taken immediately after heat treatment may also give a false impression of the efficacy of the process (Coven *et al.*, 1985) as the organisms may be in a shocked less recoverable state (Jones, 2002). The biggest under-heating problems are likely to occur during start-up periods when peak conditioning temperatures may not be reached and start-up rations should ideally be reworked. Older open kettle and ripener systems operating at lower temperatures are extremely prone to *Salmonella* contamination and are now rarely used. Closed ripeners are better (Stott *et al.*, 1975; Buick, 2000) but further heat treatment is usually necessary to ensure a *Salmonella*-free product. A combination of expansion followed by pelleting gives the best control of contamination but adds capital cost and complexity to the process (Davies and Wray, 1997).

Anaerobic pasteurisation systems (Ekperigin *et al.*, 1990), and extruders and expanders (Crane *et al.*, 1972; Sreenivas, 1999) allow higher temperatures of up to 170° C to be achieved and good bacterial reductions but some types may be difficult to stabilise. There may also be more protein and amino acid degradation at these higher temperatures (Jones *et al.*, 1995). The effectiveness of heat treatment is highly influenced by the quality of the steam used (Cooke, 2002) and this in turn depends on the design and throughput of the conditioning equipment with direct-fired steam conditioners producing hotter steam with less excess moisture (Blank *et al.*, 1996).

Experimental work has shown that prior exposure to stresses such as alkaline or acid conditions (Humphrey, 1990; Humphrey *et al.*, 1991; Farber and Pagotto, 1992; Humphrey *et al.*, 1993), dehydration (Mattick *et al.*, 2000), prior heat or cold shock (Bunning *et al.*, 1990; Mackey and Derrick, 1990; Xavier and Ingham, 1997) or starvation (Wesche *et al.*, 2005) or high mineral salt concentrations or desiccation (Palumbo *et al.*, 1995; D'Aoust *et al.*, 1997; Bell, 2002; Bacon *et al.*, 2003) may in some cases, but not all, increase the heat resistance of organisms (Casadei *et al.*, 2001) or interfere with their detection (Mackey and Derrick, 1982). The most dramatic of these effects was found after *Salmonella* Typhimurium was experimentally habituated to extreme dehydration when the organism was able to survive 60 minutes at 100° C (Kirby and Davies, 1990). It is uncertain what relevance these observations have to *Salmonella* in a natural environment such as feed but the D-value at 71° C, which would measure in seconds in a liquid medium, varies between 4.5 and 6.6 hours depending on serotype (Bell, 2002) and prior exposure to alkaline conditions may increase the susceptibility of *Salmonella* and VTEC O157 to heat (Teo *et al.*, 1996). All these variables have produced doubts about the representativeness of experimental data compared with industrial-scale treatment of complex compound feeds containing a wide range of ingredients (D'Aoust *et al.*, 1994).

There appears to be some variability amongst different *Salmonella* strains concerning their resistance to heat but some of this may be accounted for by experimental variability, different

growth stages of organisms used and in some cases use of the atypically heat resistant *Salmonella* Senftenberg 775W strain (Liu *et al.*, 1969), although *S.* Senftenberg is a common feedmill and hatchery contaminant (Davies and Wray, 1994).

It is possible to heat treat meals but this is not so easy as using a pelleting process, and subsequent cooling systems are more likely to become contaminated in the same way as many vegetable oil seed residue meal coolers (Davies and Hinton, 2000). In one experiment in which extrusion was used to decontaminate meal containing *S.* Typhimurium by seven seconds treatment at 83^{o} C at 28% moisture levels (Okelo *et al.*, 2006). Such results may be dependant on the sensitivity of detection methods however. Extrusion followed by pelleting provides enhanced control of *Salmonella* with 80^{o} C providing 10^{3} – 10^{4} reduction, and 90^{o} C giving 10^{5} reduction in highly contaminated feed (Israelsen *et al.*, 1996). A study which compared the prevalence of *Salmonella* in broiler flocks fed either on feed pelleted between 60 – 80^{o} C and other rations pelleted at 80 – 82^{o} C showed a significant reduction in the occurrence of *Salmonella* in the flocks fed the more intensively heated pellets and demonstrated that feed was the most important source of *Salmonella* for these flocks (Voeten and van de Leest, 1989). Similar findings were noted in other field studies (Vaughn *et al.*, 1974). Improved monitoring leading to a high level of control of contamination of imported feed ingredients and domestically produced compound feedingstuffs also significantly reduced the number of *Salmonella* outbreaks in animals in Sweden (Malmqvist *et al.*, 1995). Heat treatment of pelleted feed was also shown to be protective against introduction of new *Salmonella* serotypes into pig farms (Ghosh, 1972), but pelleted and heat treated feed may increase the susceptibility of pigs to *Salmonella* from other sources.

A comprehensive review of earlier work on heat treatment of animal feeds is provided by (Williams, 1981), but these findings are unlikely to be applicable to modern large-scale feed production. Further studies carried out in a standardised way in a range of commercial situations in various conditions are needed to supply useful results for quantitative risk assessment modelling (Mattick *et al.*, 2001a,b).

Short term decontamination procedures such as heat treatment or irradiation, have no residual effect so feed can be readily re-contaminated after treatment. Decontaminated feed may theoretically be more susceptible to contamination by pathogens than its original ingredients because of the reduction in indigenous flora, including potentially protective elements such as yeasts. Heat treated feed may be particularly vulnerable since the combination of warmth and additional moisture introduced into the feed during the treatment phase may promote the multiplication of organisms which may re-contaminate the milling equipment such as auger systems, pipes, storage bins and, in particular, cooling equipment which are subject to warmth and humidity from the treated feed. This may in turn re-contaminate the feed after treatment, e.g. during the cooling stage when heat treated pellets or meals are gradually cooled by forced air blast. Condensation associated with warm treated feed may be a seasonal problem, with greatest problems in the autumn, when there is a wide fluctuation between day-time and night-time temperatures. Storage bins located on the coldest side of the mill in relation to prevailing winds may also be subject to more condensation. Control of condensation is variable according to the conditioning temperature and the time between conditioning and cooling as well as the effectiveness of the cooling process and insulation and air movements in storage areas. In some cases feed may still be warm on delivery to the farm in which case condensation may occur in farm storage bins. Equipment used for cooling may become transiently or persistently contaminated by *Salmonella* as a result of intake of contaminated cooling air or passage of feed which has been incompletely heat treated. In mixed species mills feed intended for ruminants or pigs is normally conditioned at lower temperatures than poultry feed and this may serve as a source of contamination of the manufacturing line and subsequent contamination of poultry feed produced on that line. In situations when the post heat treatment feed processing

equipment does become persistently contaminated by *Salmonella* it is common to find that finished products are more likely to be contaminated than the ingredients used to manufacture the feed (Forshell and Svedberg, 1983; Davies, 1992; Davies and Wray, 1997; Davies and Hinton, 2000; Davies *et al.*, 2001a) but may still be missed because of the limitations of sampling sensitivity. Many of these endemic contaminating serotypes of pellet cooling systems may subsequently be found in poultry flocks consuming the feed and poultry products derived from the birds (Corry *et al.*, 2002). International dissemination of feed-related *Salmonella* serotypes should also be considered since imported foodstuffs may in some cases appear to pose a greater threat to public health than indigenous strains (Threlfall *et al.*, 2003). In conclusion, effective heat treatment of feed to eliminate *Salmonella* is possible but adds cost. It is also necessary to safeguard the treated feed against recontamination.

8.2.1.1. Effect of feed heat treatments on organisms other than *Salmonella*

Although reduction of *Salmonella* in feed is the primary objective of any treatment it is useful to be able to predict the likely effect of these treatments on other organisms which may be of public health concern. Predictive modelling can be useful for this but there is a need for good quality data and careful interpretation for this to be reliable (Valdramidis *et al.*, 2005; Buzrul and Alpas, 2007). The most common cause of foodborne zoonoses in most countries is *Campylobacter*. This survives poorly in dry conditions exposed to air and has a lower resistance to disinfection and heat than *Salmonella*. It would therefore be expected that processing conditions capable of containing *Salmonella* contamination would also be suitable for many foodborne pathogens (Sörqvist, 2003) including *Campylobacter*, even though feed is not considered to be significant in the epidemiology of this organism (Newell and Fearnley, 2003).

VTEC O157 and *Listeria monocytogenes* occur commonly in ruminants and less commonly in other animals. Direct faecal contamination of the environment of the animals is the major route of spread, although *Listeria monocytogenes* and other organisms may survive at high levels or even multiply in poorly fermented (i.e. not achieving a sufficiently low pH) silage which is subject to contamination by soil during harvesting (Chen *et al.*, 2005). There are far fewer reported studies of these organisms in feed than have been reported for *Salmonella* but some information if available. In beef heated to 55–60^{o} C D-values for *Listeria monocytogenes* were significantly higher than for *Salmonella*, VTEC O157 or most indigenous microflora (Juneja, 2003), although similar heat resistance profiles were observed in heated liquids (Huang, 2004). A subpopulation of microflora showed higher levels of heat resistance, and these could therefore be used as indicator organisms to assess the success of heat treatments (Juneja, 2003). *Enterobacteriaceae* counts can also be used for assessing the effectiveness of heat treatment in the animal feed pelleting process (Coven *et al.*, 1985). The frequent occurrence of faecal enterobacteriaceae in feed may also provide a means of international dissemination of antimicrobial resistant organisms, e.g. ESBL producers.

Another study suggested that *Bacillus brevis* could safely be added to feed to act as an indicator organism (Cooke, 2002) and that heat processing conditions which were appropriate for elimination of *S.* Senftenberg and *S.* Anatum (i.e. D-value at 80^{o} C at 0.8 Aw – 12.3 minutes) could also eliminate other *Salmonella* serotypes including *S.* Enteritidis (6.9 mins), *S.* Typhimurium DT104 (7.7 mins), as well as *Listeria monocytogenes* (5.4 mins), *Yersinia pseudotuberculosis* (<0.3 mins) and *E. coli* O157:H7 (6.3 mins). Other studies applying heat treatment to beef, chicken meat and skin also found *L. monocytogenes* was more easily eliminated than *Salmonella* (Juneja, 2003; Murphy *et al.*, 2004) but reduction profiles for *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 were similar in meat sausages, but *Listeria* was more heat sensitive than *Salmonella* in turkey Bolognese (McCormick *et al.*, 2003). Studies of on-farm pasteurisation of milk suggested that conditions capable of eliminating

Salmonella could also control *Mycobacterium paratuberculosis* and *Mycoplasma* (Stabel *et al.*, 2004) but recovery of *M. paratuberculosis* is difficult and may have underestimated survival. Steam pasteurisation of beef for 15s at 87.8^{o} C proved to be ineffective for *Salmonella* Typhimurium, *E. coli* O157:H7 and *Listeria innocua* but 98.9^{o} C for 9s produced >3.5 log reduction in all pathogens (Retzlaff *et al.*, 2004). *Listeria monocytogenes* appeared to be controlled more effectively than *Salmonella* by commercial heat treatment of mash feeds, especially when a direct final steam system was used (Blank *et al.*, 1996) but in another study *L. monocytogenes* was more resistant than *Salmonella* in egg yolk (Palumbo *et al.*, 1995). *Listeria monocytogenes* appears to be less susceptible to increased heat resistance after prior acid or heat shock than *Salmonella* (Bunning *et al.*, 1990; Farber and Pagotto, 1992).

Recently results have been obtained which suggest that the heat resistance of *E. coli* O157:H7 in concentrate cattle feeds may be greater than that of *Salmonella*, in that heating at 70^{o} C for 120s only resulted in up to 2.2 log reduction with the worst results in higher fat feeds, but this was carried out in a small scale model system and no direct comparison with *Salmonella* was carried out (Hutchison *et al.*, 2007). Since VTEC O157 is already widespread in ruminants, feed is not regarded as a primary route of spread but contamination of feed may be relevant for poultry and pig populations which are otherwise protected from contact with ruminant faeces.

In conclusion, heat treatment conditions which are effective for eliminating the risk of *Salmonella* acquired from feed would be expected to also effectively control other major food-borne zoonotic organisms. More work is required to define the limitations and risks associated with acid tolerant organisms when feed is acidified

8.2.2. Chemical treatment of feedstuffs

Numerous compounds have been cited as possible means to control contamination by *Salmonella* and other undesirable microorganisms (Smyser and Snoeyenbos, 1979). These include acetic acid, propionic acid and buffered propionate (Ha *et al.*, 1998), citric acid, ethanol, formaldehyde, formic acid, isopropyl alcohol, zinc acetate and zinc propionate (Martin and Maris, 2005; Ricke, 2005). The practical usability and efficiency of these agents varies widely (Skrivanova *et al.*, 2006) but medium chain fatty acids have a greater effect on *Salmonella* than short chain fatty acids (van Immerseel *et al.*, 2002) and zinc acid salts may be more effective than sodium salts (Park *et al.*, 2003), and it is difficult to obtain definitive data on their likely performance in field situations as most work is based on small scale artificial contamination experiments using recent broth cultures rather than naturally dormant contaminants or on uncontrolled observational studies. Antibacterial feed additives should be should be stable to the point of consumption but either metabolised or not absorbed so there are no residues in meat, milk or eggs from animals consuming the treated feed.

Legislative framework in terms of Community legislation: Preservatives are defined in Regulation (EC) No 1831/2003 on feed additives as substances or, when applicable, micro-organisms which protect feed against deterioration caused by micro-organisms or their metabolites. If chemicals are used for other purposes than feed additives e.g. as biocides the respective legislation would have to be considered

8.2.2.1. Acidification-based treatments

It is thought that the primary antibacterial effect of organic acids is due to their ability to disrupt pH gradients and intracellular pH regulation, which in turn leads to interference with other chemical processes (Cherrington *et al.*, 1990; Cherrington *et al.*, 1991a; Cherrington *et al.*, 1991b; van Immerseel *et al.*, 2006). Acids may also interfere with the expression of virulence genes and so reduce intestinal invasion by *Salmonella*, although habituation after prior exposure to acids may occur (de Jonge *et al.*, 2003; Greenacre *et al.*, 2006; El-Sharoud and Niven, 2007) which may facilitate invasion and survival within macrophages (Kwon and

Ricke, 1998). Development of a tolerance response to low pH conditions may decrease the susceptibility of *Salmonella* to strong acids but still leave them vulnerable to the direct toxic effects of weak organic acids (Baik *et al.*, 1996). There are fears that selection for acid tolerant organisms by use of organic acid feed treatments or feeding regimes which promote a low pH may lead to development of clones of *Salmonella* or VTEC which are more likely to survive gastric acidity in humans consuming food contaminated by such organisms (de Jonge *et al.*, 2003; Fratamico, 2003; Theron and Lues, 2007).

Chemical decontamination may not always be as effective as a suitable intensity of heat treatment but it can provide some residual protection against recontamination (Rouse *et al.*, 1988; Carrique-Mas *et al.*, 2007), depending on the product used, the application rate, thoroughness of application (Rejholec, 1980), and persistence (Hinton and Linton, 1988; McCubbine, 1989). There is also some evidence that some of the products may promote an unfavourable intestinal environment for colonisation of animals by *Salmonella* originating from other sources, such as the environment, thus limiting the within-flock prevalence of infection and disease and the numbers of organisms excreted in faeces or contaminating processed carcasses (Hinton and Linton, 1988; Humphrey and Lanning, 1988; Thompson and Hinton, 1997; Al-Tarazi and Alshawabkeh, 2003; Al-Natour and Alshawabkeh, 2005). This reduction may be achieved despite what appears to be incomplete control of contamination of feed in its dry state (Duncan and Adams, 1972; Vanderwal, 1979; Banton *et al.*, 1984; Dunn, 1987; Hinton and Linton, 1988; Humphrey and Lanning, 1988). This is because the acidic products at the application rates used may be largely bacteriostatic (Carrique-Mas *et al.*, 2007) and further reduction in *Salmonella* may be achieved after consumption of the feed, when it is rehydrated by saliva and gastric fluid (Busta *et al.*, 1976; Hinton and Linton, 1988; Cherrington *et al.*, 1991a; Albuquerque *et al.*, 1998), so that organisms which are dormant in feed are killed as they resume multiplication. Acidified feed is most effective for limiting *Salmonella* if it is given throughout the rearing or production period since its effects are limited once animals have become infected and *Salmonella* microcolonies have become established in gut-associated lymphoid tissue and mesenteric lymph nodes, where no contact with intestinal acids is possible, and in the caecum, in which case most of the acidic additives have already been metabolised (Hume *et al.*, 1993; Hume *et al.*, 1993). The efficiency of organic acid feed treatments varies widely with the type of acid or blend of acids, the physical nature of the product used, i.e. whether applied as a liquid or granules (Duncan and Adams, 1972), the inclusion rates (Thompson and Hinton, 1997), and whether the products are present as free acids or acid salts (which may limit contact between the target organisms and the treatment) and the level of *Salmonella* in the feed. In general the level of efficiency can be increased by increasing the level of acids used or by using liquid products with a high proportion of free acids. Despite this, reduction of *Salmonella* in feed after acid treatment may take several days to achieve its full effect (Vanderwal, 1979) and feed may have already been fed to birds within hours of production (Vanderwal, 1979; Staden *et al.*, 1980; Hinton and Linton, 1988). A proportion of feed may also pass very rapidly through the crop so that the exposure time to acids after rehydration of the feed may be minimal (Hume *et al.*, 1993; Hume *et al.*, 1993) and viable organisms may then reach the intestine leading to infection. Even if this only happens in a very small proportion of animals subsequent spread via faecal shedding by a small number of infected individuals is likely to overwhelm any ongoing protective effect of feed or other treatments. High levels of acidification may have adverse implications in terms of cost, corrosiveness for milling and feeding equipment, safety of feed production workers, and palatability (Pinchasov and Jensen, 1989) or interfere with availability of vitamins (Rys and Koreleski, 1974; Cave, 1984). Intensive high-level acid treatment may therefore be more suitable for treatment of batches of highly contaminated ingredients prior to mixing (Malmqvist *et al.*, 1995). This approach also provides a lower level treatment which may be beneficial for the compound feedingstuffs which incorporates the treated ingredient.

There is a wide variety of organic acid products which are commercially available for treatment of animal feeds. Most products are blends of short-chain organic acids such as formic acid and propionic acid but acetic acid and butyric acid may also be used (Matlho *et al.*, 1997; Thompson and Hinton, 1997; Ricke, 2005). Achieving the most effective blend is most important and variations in formulations, as well as challenge, may explain the variable results obtained in different trials (Luckstadt, 2005). Organic acids have also been used for food preservation and inhibition of moulds and spoilage organisms in human foodstuffs and silage for cattle and sheep (Baird-Parker, 1980).

The bacteriostatic effects of organic acids are considered to be partially related to lowering of pH in the feed matrix and in the proximal parts of the digestive tract of animals consuming the feed (van Immerseel *et al.*, 2002; Ricke, 2003) but the antibacterial effect of organic acids is greater than the effect of mineral acids at the same pH and activity varies between the different acids so there is also likely to be a direct toxic effect. There may also be varying buffering effects exhibited by different protein feed ingredients on different acids (Tabib *et al.*, 1984). Such antagonistic effects may be minimised or rapid metabolism of acids slowed down by use of carrier products or micro-encapsulation (Iba and Berchieri, 1995; van Immerseel *et al.*, 2004b; van Immerseel *et al.*, 2005).

Acidification may also have a beneficial effect on the balance of commensal intestinal organisms, even when the acids are applied on litter (Garrido *et al.*, 2004) or in drinking water (Chaveerach *et al.*, 2004). This may lead to a partially protective effect against various potential intestinal foodborne pathogens such as *Salmonella* and *Campylobacter* (Heres *et al.*, 2004) although this effect may be inconsistent (Izat *et al.*, 1990; Hume *et al.*, 1993; Hume *et al.*, 1993). Other undesirable intestinal organisms such as pathogenic *E. coli* and *Clostridium perfringens* may also be reduced by acidification of feed, although the antibacterial effect varies widely between bacteria (Russell and Diez-Gonzalez, 1998; Martin and Maris, 2005). Organisms such as VTEC O157 and *Listeria monocytogenes* may be relatively acid tolerant (Diez-Gonzalez and Russell, 1997) so may not be affected by organic acids at normal feed treatment levels. General microbial contamination of broiler carcasses may be reduced by feeding acidified rations (Aksit *et al.*, 2006). Beneficial organisms such as lactobacilli are highly acid tolerant so may be favoured by feed acidification (Hsiao and Siebert, 1999) and this beneficial effect on intestinal flora may have a growth performance enhancing effect which may help offset the cost of the treatment (Leeson *et al.*, 2005; Diebold and Eidelsburger, 2006). This effect may only be seen under sub-optimal management conditions (Hernandez *et al.*, 2006). There may also be beneficial effects in terms of control of the development of moulds and production of fungal toxins in treated feed (Paster *et al.*, 1987; Brake *et al.*, 1990).

8.2.2.2. Formaldehyde-based feed treatments

According to the feed additive legislation, Formaldehyde is only authorised at Community level as preservative for skimmed milk for pigs up to the age of six months and for all species or categories of animals as silage additive.

Scientific studies have demonstrated that formaldehyde has a high level of disinfectant activity against most bacteria and is the most effective compound to use for disinfection of poultry houses which have been contaminated by *Salmonella* (Davies and Wray, 1995). It is less likely to be inactivated by organic matter than most disinfectant classes, but the action of formaldehyde is slow compared to some less effective disinfectants, requiring several hours to achieve its full effect. Various studies have demonstrated superior decontamination of feed by formaldehyde compared with acid products (Duncan and Adams, 1972; Smyser and Snoeyenbos, 1979; Moller, 1983; Moustafa *et al.*, 2002). Some commercial products contain a blend of formaldehyde, propionic acid and other dispersing agents. This combination has been shown to achieve greater decontamination of feed which has been artificially inoculated with

Salmonella compared to various acid products (Carrique-Mas *et al.*, 2007). In this study there also appeared to be less masking of viable organisms by the treatment than with acid products when the feeds were tested after neutralisation of the treatments. It is possible that the partially protective effect of formaldehyde treated feed against acquisition of low levels of *Salmonella* from other sources is less than what can be achieved with a good organic acid blend (Cherrington *et al.*, 1991b) although other studies do suggest a beneficial effect (Mone, 1987).

The long-term protective effect of formaldehyde may be limited to some extent by evaporation after mixing, unless feed is held in closed bins (David *et al.*, 1972; Khan *et al.*, 2003). For this reason some commercial formaldehyde-based products also contain acids such as propionic acid and other antimicrobial compounds such as terpenes (Trombetta *et al.*, 2005; Carrique-Mas *et al.*, 2007). This produces a synergistic combination allowing lower levels of formaldehyde and acids to be used which minimises fuming, operator hazard and corrosiveness. Such products can therefore be more readily used in feed mills than the more potent acid blends which require non-corrodible equipment and special safety procedures. Such blended products may lead to improvements in productivity in some cases but have little influence on resistance to exposure to environmental organisms (Anderson *et al.*, 2002). The product may however be useful to limit contamination of feeding systems and has been used at higher levels on a grain, wheatfeed or woodchip carrier to decontaminate the interior of inaccessible equipment (Furuta *et al.*, 1980; Torroella *et al.*, 1987) such as auger systems in feed mills or closed feed bins and pipes serving pan feeders on poultry farms.

Formaldehyde has also been used for a long time for preservation of animal feedingstuffs such as whey, silage or protein ingredients (Barker *et al.*, 1973; Bhargava *et al.*, 1979; Summers *et al.*, 1980; Morgan, 1985)and has been used to help preserve feed proteins from degradation by ruminal microorganisms and therefore improve the economy of beef and milk production (Madsen, 1982). There may however be some risk of formaldehyde passing into the milk of animals fed on high levels of the chemical (Barry and Tome, 1991).

In conclusion, chemical treatment with effective products applied correctly can be a viable alternative to heat treatment and also offer some protection against recontamination. Some acid combinations may also have beneficial effects on the health and performance of the animals. Chemical treatments can also be used for decontamination of feeds for animals such as commercial layers where heat-treated or pelleted feed may be undesirable.

8.2.3. Potential Adverse effects of processing

The intestinal tract of animals has evolved to process foodstuffs in their natural state. This involves coarse grinding and breaking down of the material by mastication, ruminal action or activity of the crop. A variety of foraged and hunted foodstuffs form a varied natural diet. The industrialisation of food animal production has resulted in the need for large-scale production of feedstuffs which promote the most rapid efficient growth or milk or egg production at minimal cost. Grinding and heat treatment of feed ingredients increases the growth rate and feed conversion efficiency and pelleting increases the intake of feed in animals when ad-lib feed intake to achieve maximum growth rate is important.

In pigs the provision of finely ground feed, heat treated feed or pelleted feed has been shown to increase the risk of detection of a significant level of *Salmonella* in the herd (Kranker *et al.*, 2001; Leontides *et al.*, 2003) whereas feeding waste feed, whey, liquid feed (especially when subject to controlled fermentation) and feeding of coarsely ground rations containing barley rather than wheat are associated with a lower risk (Beal *et al.*, 2002; Mikkelsen *et al.*, 2004; Farzan *et al.*, 2006). This means that there is a potential conflict between the desire to promote the production of *Salmonella*-free feed by pelleting and the potential perturbation of intestinal flora associated with pelleted feeds, which increases the risk of amplification of the intestinal

colonisation and multiplication of *Salmonella* which have accessed the animals from other sources.

Less is known about the effect of feed formulation on the establishment of pathogens in poultry, but it is known that the physical formulation of feed can influence volatile fatty acids, pH, and therefore microbial populations in the digestive tract of broilers (Engberg *et al.*, 2002) and that the balance of volatile fatty acids is involved in dysbacteriosis (Louis *et al.*, 2007) which may lead to poor performance, wet litter and increased use of antibiotics. Broilers receiving pelleted diets experienced a reduced caecal pH and increased levels of *S.* Typhimurium in gizzards and caeca compared with birds fed meal rations formulated from exactly the same ingredients (Huang *et al.*, 2006). Caeca were also enlarged in pellet fed birds which could result in greater risk of intestinal rupture and carcase contamination at slaughter. Birds fed whole wheat are also likely to have reduced colonisation by *Salmonella* compared with those fed pelleted feed (Bjerrum *et al.*, 2005), and many producers now feed whole wheat alongside pelleted rations in an attempt to improve 'gut health' (Bjerrum *et al.*, 2005). Partial whole wheat feeding may also be associated with reduction of *Salmonella* infection in parts of the intestinal tract and an overall reduction in numbers of *Clostridium perfringens*.

The use of pelleted feed which requires fine grinding to obtain good pellet quality, is particularly undesirable for commercial laying hens, where mashes have been used traditionally in most countries (Nelson, 2008). Pelleted rations would increase the chance of acquisition and persistence of *S.* Enteritidis from environmental sources, which occurs commonly in most countries, would produce more fluid droppings which would contribute to contamination of egg belts, litter and eggs and lead to over consumption of feed by birds leading to weight-related problems or aggression through rapid consumption of pellets followed by frustration with empty feed troughs. Moist manure is also more conducive to fly problems in cage laying systems and skin and locomotor conditions in birds in barn or free-range housing.

In contrast, potential adverse effects of organic acid treatments relate to an observed increase in invasiveness in cell culture studies, although this has not been demonstrated in live animals or in the field (van Immerseel *et al.*, 2002) and further studies are required to elucidate this. There is also a theoretical potential for promotion of acid-tolerance in micro-organisms or preferential survival of organisms which are already relatively acid tolerant which may then be more resistant to the gastric acidity barrier (de Jonge *et al.*, 2003; Fratamico, 2003; Theron and Lues, 2007).

In conclusion, the beneficial effects of treatments to eliminate *Salmonella* normally outweigh any disadvantages, but exceptions may apply in the case of heat treatment and pelleting for feed intended for animals regularly exposed to *Salmonella* from other sources.

8.2.4. Alternatives to heat or acid treatments

Irradiation has been used for treatment of small consignments of feed for specific pathogen free (SPF) laboratory animals and herbs (Mossel *et al.*, 1967; Epps and Idziak, 1972). 10-40 kgray completely eliminated all microorganisms and insect pests in feed without any adverse effects on birds consuming the feed, or radioactive residues (Leeson and Marcotte, 1993). Despite the effectiveness and energy efficiency of this method, because of cost considerations and public opinion this treatment is unlikely to be adopted routinely for animal feed in the foreseeable future.

High pressure treatment can be used to disrupt the integrity of microorganisms and to promote an antimicrobial effect either alone or in combination with other treatments such as heat (Yuste *et al.*, 2000; Teo *et al.*, 2001; Wuytack *et al.*, 2003; Malicki *et al.*, 2005). These approaches appear to offer promise and should be investigated further, although the capital costs to develop and install equipment for high throughput production would be large.

Microwave energy can also be used (Heddleson and Doores, 1994; Heddleson *et al.*, 1996; Lagunas-Solar *et al.*, 2005) but it seems unlikely that this could be developed for industrial feed production in the foreseeable future.

8.2.5. Use of indicator organisms as a process control for animal feeds

Because of the sporadic nature of *Salmonella* contamination, most tests carried out for monitoring purposes are negative. This provides helpful epidemiological information when those *Salmonella* which are identified have been typed and compared with trends in animals and humans. However, a series of negative results does not assist with monitoring the general antimicrobial effect of processes applied (Cooke, 2002; Sperber, 2007). It is therefore valuable to monitor also indirect indicators of extraneous contamination such as aerobic plate counts, enterobacteriaceae counts, or *E.* coli (the latter two being indicative of faecal contamination), moulds, or yeast counts (Sperber, 2007). Of these, enterobacteriaceae counts are probably the most meaningful and simplest to apply (Gradel *et al.*, 2003; Jones and Richardson, 2004). A harmless indicator organism may also be added and assessed before and after treatments (Cooke, 2002). More work is required to define a validated standardised approach to the use of indicator organisms in feed production, either as a means of assessing faecal contamination of ingredients and compound feedingstuffs, or to assess quantitative reductions in contamination produced by heat or chemical treatments. The level of indicator organisms is however not always linked with the risk of *Salmonella* contamination, e.g. *Salmonella* originating from oilseed residue or pellet cooling systems may be present regardless of the microbiological status of the feed in terms of faecal indicators or other organisms. It is therefore not desirable to designate microbiological criteria for indicator organisms but it is appropriate to refer to the voluntary use of microbial counts according to requirements of feed manufacturers.

8.3. Feeding strategies to control Salmonella

This chapter is dedicated to the measures and procedures that aim at preventing or reducing or limiting the multiplication, colonisation and penetration of *Salmonella* in the gut. Prebiotics such as monosaccharides, disaccharides and polysaccharides have complex effects which may exert some direct actions on pathogens, but the greatest effect is likely to be on the development of the indigenous intestinal flora which may favour organisms which are antagonistic to pathogens such as *Salmonella* and VTEC O157. This is a complex area which is outside the remit of this opinion.

8.3.1. Rough grinding and potassium diformate

In recent years, the effect of the particle size of feed for pigs on the morphological characteristics of the small intestine and its potential to reduce the adhesion of *Salmonella* to the epithelial cells was discovered (Mikkelsen *et al.*, 2004; Hedemann *et al.*, 2005; Papenbrock *et al.*, 2005). Additionally, the use of potassium diformate as feed additive for growth promotion (Windisch *et al.*, 2001) was described as having the capability of reducing the shedding, since the amount *Salmonella* Derby shed by experimentally infected pigs was significantly reduced (Papenbrock *et al.*, 2005). The combination of both (coarse grinding and potassium diformate) has been increasingly used in the last two years in the framework of the German *Salmonella* monitoring and reduction programme (within the so-called "QS-System"). Together with hygienic measures at farm level, the combination of the two measures has led in many herds to a gradual decrease of the number of pigs with *Salmonella* antibodies. Exact epidemiological data are not yet available, but will be generated I the further course of the national *Salmonella* control programme in Germany.

8.3.2. Liquid feeding

In the end of the 90's of last century, epidemiological data from The Netherlands (van der Wolf *et al.*, 1999) and data from the assessment of the *Salmonella* control programme in pig and pork production that Denmark had implemented in 1995 pointed to the fact that herds with liquid feeding seemed to be remarkably less frequently categorised as *Salmonella* positive herds than those that were fed with pellets (Kranker *et al.*, 2001). The following systematic investigation of this assumption led to data that clearly demonstrate the association between the type of feeding (liquid or pellets) and the probability to belong to the category of herds with the highest *Salmonella* load (Lo Fo Wong *et al.*, 2004; Farzan *et al.*, 2006). The mechanism of the effect of the liquid feeding on the amount of *Salmonella* in the herd is not completely understood, but the assumption that pellets have too rapid a passage through the stomach, and thus, too little acidification is plausible.

8.4. Examples of effectiveness of reducing Salmonella contamination under industrial scale.

Few data are available on the effectiveness of heat treatment and subsequent steps in the feed chain for the elimination of *Salmonella* under industrial scale, probably because such studies requires in dept studies that often are difficult to perform. However, long term (1994-1997) data are available from a Norwegian crushing plant producing of soy meal from soybeans imported from South America where the plant applies the methods for prevention of *Salmonella* contamination as suggested in Chapter 9. In spite of an initial high contamination of *Salmonella* (approximately 30%, Appendix A, Figure I) generally no *Salmonella* is detected from the soy meal produced. Similar results are found when the meal is exported to Sweden, where as a national demand, it is tested as feed ingredients before introduction to feed producing plants (Wierup, 2006). Long term studies from a crushing plant for rape seed from European countries has also demonstrated that rape seed meal can be produced without detectable contamination of *Salmonella* (Herland, 2006). A corresponding example for the effectiveness of currently available methods the prevention of *Salmonella* contamination of feed is available from the production of chickens in Sweden where a stringent control of *Salmonella* in feed and animal production since long is applied (EFSA, 2006a). Apart from the data from the *Salmonella* control of the feed factories the result of the *Salmonella* control in poultry can be used as an additional indicator. Commercially hatched chickens are thus known to be very susceptible also to exposure low doses of *Salmonella* (Schneitz and Mead, 2000). The very low detecting rate of *Salmonella* in those tests (annually is tested approximately 3,500 flocks of 20,000 animal per flock) together with similar experience from the control of *Salmonella* in the slaughter chicken production in e.g. Finland, Norway and Denmark (EFSA, 2006a) demonstrates the effectiveness of the currently available methods for the control of *Salmonella* also in compounded feed. In summary, long term studies demonstrate that methods are available and in use under industrial scale for the production of *Salmonella* free feed material as well as of compounded feed in spite of an often relatively high initial *Salmonella* contamination.

It is also interesting to note that certain feed, in particular feed to be delivered to top breeding poultry flocks and to meat producing poultry flocks often are reported to have a lower prevalence of *Salmonella* contamination than feed e.g. cattle. This most likely is a result of those greater efforts being made to ensure freedom from *Salmonella* contamination in respond to the greater demand for *Salmonella* free feed to the sensitive production animals, efforts which thus are proven to be effective. The industry based data from 2005 and 2006 (Anon., 2007a) reports an incidence between 0 and 0.8% of *Salmonella* contaminated samples in compounded feed to different food animal species (poultry, swine and cattle). The lowest prevalence was found in feed for top breeding poultry flocks. Feed were supplied to such flocks

first following it has been analysed and found *Salmonella*-negative. Meanwhile the feed was kept in store for a week (quarantine) while awaking the result of analysis. In Sweden was also verified that mills delivering poultry feed usually applied a significantly more stringent monitoring than applied for feed to other animal species (Wierup, 2006).

9. Microbial testing of feed and establishment of microbiological criteria for *Salmonella* in feed

9.1. General considerations related to microbial testing.

It is recognised that no practical sampling (time and money) plan can ensure absence of the target microorganism in feed. As previously mentioned (chapter 6), confidence in the results of testing will depend on the number of samples units tested, whether or not there is a homogeneous distribution of the target organism in the lot and whether the sampling is performed randomly. In addition the sensitivity and specificity of the used testing method has to be taken into account. In general, it is not appropriate to perform only microbiological testing of end-products and in general ensuring safety by end-product analysis alone is simply not possible in a cost-effective manner. For these reasons microbial testing of end-products is only one of several methods used to control the occurrence of pathogens in food/feed and this has to be used only as an integrated part of a HACCP-based control system (see Chapter 8) together with sampling and testing earlier and when applicable also later in the production chain.

9.2. Background: Microbiological criteria as defined in the EU legislation

An EU Food Safety Criterion defines the acceptability of food products. If the criteria are not met the product / batch must not enter the market, and it has to be withdrawn if it has been placed on the market. An EU Process Hygiene Criterion gives guidance on, and is an indicator of, the acceptable functioning of HACCP-based manufacturing, handling and distribution processes. It sets indicative contamination values above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law.

EC Regulation 2160/2003 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0001:0015:EN:PDF) on the control of *Salmonella* and other specified food-borne zoonotic agents, aims to ensure that proper and effective measures are taken to detect and control *Salmonella* and other zoonotic agents at all relevant stages of production, processing and distribution, including in feed, in order to reduce their prevalence and the risk they pose to public health. Those specific requirements should be based on targets for the reduction of the prevalence of these agents in animal populations, mainly at the level of primary production and, where appropriate at other stages of the food chain, including in food and feed and in accordance with Article 5(3) of Regulation (EC) No 183/2005 on feed hygiene specific microbiological criteria and targets shall be adopted.

Due to the statistical limit of sampling plans, microbiological testing of food and feed for pathogens occurring at a low prevalence, may give a false feeling of safety if not a sufficient number of samples are tested over time. Most food safety criteria are based on two class sampling plans with 5 or 10 units tested per sample, except for infant formulae where 30 units should be tested (EC Regulation 2073/2005, http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_338/l_33820051222en00010026.pdf). Therefore, for pathogens present in food lots at a low frequency, the risk of not detecting contaminated food lots is high (ICMSF, 1986). In these cases efficiency of applying food safety criteria on food/feed to improve animal and consumer protection will be low.

9.3. Microbiological Criteria for Salmonella in feed

Because of the possibility of infection of animals with *Salmonella* the objective should be that this organism should not be present in animal feed. In different surveys *Salmonella* are detected in compounded feed in low prevalences (Chapter 4). However, due to the limitations of available methods for detecting a low prevalence contamination (Chapter 9.1), this makes the efficacy of establishment of a feed safety criteria based only on the end product questionable. A feed safety criteria based only on testing of the end product would also as a routine procedure require storage of the feed during the testing procedure before delivery.

A more efficient option could be the establishment of one or more process hygiene criteria at certain critical stages in the production chain including in the end product. When considering that the prevalence of *Salmonella* contamination in certain feed ingredients is found to be high and identified as a risk for contamination of the subsequent feed chain (Chapter 4), the objective should be to prevent the *Salmonella* contamination as early as possible in the feed chain. Such approach is in line with the EU´s recently adopted Animal Health Policy (2007-2013) that states "Prevention is better than cure".
(http://ec.europa.eu/food/animal/diseases/strategy/index_en.htm)

9.4. Microbial testing and establishment of process hygiene criteria as an integrated part of individual HACCP-based programmes

According to the feed hygiene directive 183/2005 (http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2005/l_035/l_03520050208en00010022.pdf) feed businesses should meet several detailed conditions relevant to their operations concerning facilities, equipment, personnel, production, quality control, storage and documentation. In 183/2005 it is stated that feed business operators shall put in place, implement and maintain a permanent written procedure or procedures based on the HACCP principles. In addition they must implement effective monitoring procedures and establish corrective actions when monitoring indicates that a critical control point is not under control.

Testing for *Salmonella* along specified places in the production line and testing for *Salmonella* in the end product should be an integrated part of an efficient HACCP-programme. All serotypes of *Salmonella* should be equally treated and the isolation of *Salmonella* should be notified.

Ideally, preventive action should start at the level of primary production. In the lack of such experiences for the prevention of *Salmonella* contamination during the cultivation primarily of oil seeds but also for other crops, the control should be directed in particular to the crushing premises processing these products. For the animal derived products prevention to a varying extent is already in place during the primary (animal) production, but has to be focused also on the rendering plants. The control at these premises, crushing and rendering plants, should when applicable follow those steps that below are described for the feed mills. The importance of starting the control already at the crushing and the rendering plants is emphasised in contrast to the currently often applied practice to focus only on the feed mills.

9.4.1. Testing of incoming raw material

Feed material could be classified according to risk for being contaminated by *Salmonella* (Chapter 4.1.2) and feed material identified as risk products should be monitored before intake. Animal and fish derived protein including e.g. meat and bone meal (MBM) and fish meal as well as vegetable proteins derived of soya bean, rape seed meal and palm kernel could be regarded as high risk products. However, the risk for *Salmonella* contamination of feed material may vary between different Member States. Depending on the processing applied the risk may also vary between several of those feed materials that often are derived from the same

product, as presented in Commission Directive 98/67/EC which presents a non exclusive list of feed materials put into circulation.

The monitoring of batches of feed material has the same limitation for the probability of detection of *Salmonella* as for monitoring of compounded feed as described earlier. Therefore the sampling in practice can only be designed to exclude the introduction of highly contaminated lots. Sampling according to (Ekbohm, 1993) could be applied which take into consideration an uneven distribution of *Salmonella* contamination. The number of samples per lot may vary within the group of identified feed materials depending on the risk for *Salmonella* contamination. For instance one 50g sample of each major ingredient taken from a single batch (i.e. not a composite sample) and as representative as possible, ideally using an automated in-line sampling device designed to detect contamination in 5% of the batch with 95% confidence, could be taken. In the case of compound feed mills this sampling will include major cereal ingredients as well as protein ingredients.

The actions to be taken if lots of ingredients are found positive for *Salmonella* will depend on the specific process that follows, and the specific HACCP programme but may include i.e. rejection, decontamination, and contact to producer etc.

9.4.2. Testing at key sampling points along the compound feed production chain

The following places along the production chain are normally regarded as key sampling points in feed mills for production of compound feed and when applicable also in crushing and rendering plants:

(i) Unloading pit for feed materials – ideally sample dust escaping from the elevator which removes feed materials from the pit. If this is not accessible sample accumulated dust from multiple areas within the pit area

(ii) Ingredient sieve, or aggregate samples of dust from within ingredient bins

(iii) Filter aspirating the production line – this may only be accessible during stoppage of production, or may not be suitable in small mills or those which discharge directly to the exterior of the mill. Sample accumulated dust in the aspiration system or its collection bins

(iv) Pellet or meal cooler – collect dust emanating form the coolers. If this is positive follow-up by sampling aggregate at the entry point for pellets or meal inside the cooler

(v) Pellet shakers and crumblers – take dust escaping from machines

(vi) Dust within finished product bins or when not present or collectable accumulating below or on outloading gantries. In smaller mills, dust from bagging plants

Samples of dust from these places should be tested for *Salmonella* at regular intervals as part of an efficient HACCP-based control programme, in Sweden testing at one week intervals has been found appropriate.

If samples are tested positive for *Salmonella* corrective actions should be taken. Depending on the place of finding these should include, cleaning and disinfection, increased monitoring, stop of production and stop for delivery of compound feedingstuffs. Stop of production and delivery of compound feedingstuffs is especially important if *Salmonella* is found in top of pellets cooler and in top bin of compound feedingstuffs.

A common EU process hygiene criteria for *Salmonella*, in the production chain at one or more of the above key sampling points, could be considered.

9.4.3. Testing of compound feed

Testing of compound feed for *Salmonella* should be used as part of an integrated method to validate the efficiency of the HACCP based control program. An overall requirement should be that the final feed is free from *Salmonella*. Daily samples could be taken as near the point of despatch from the premises as possible for each product category. Samples could i.e. be bulked by category into aggregates and i.e. 50 g samples could be tested at specific intervals i.e. weekly. The samples should be taken in a way that maximises their representativeness of the batch, ideally using an automated in-line sampling device.

If samples are tested positive then corrective actions should be taken including i.e. (i) Investigation of the particular sections of the plant through which the product was manufactured paying particular attention to those Critical Control Points at which *Salmonella* contamination is most likely to occur, (ii) Investigation of raw material records appropriate to the compound feedingstuffs sample, (iii) Application of an effective treatment regime for feed produced (iv) Increased intensity of sampling and testing of production.

It is suggested that a common EU process hygiene criteria for compound feedingstuffs is established according to what is described above.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Forage, industrial compound feed, home-grown cereals and purchased straight feedingstuff are the four major groups of feeds for EU livestock. Only 5.7% of cereals are imported from third countries. In contrast, the EU self-sufficiency is relatively low for protein-rich feed materials (except for fish meal), and as low as 2% for soy bean meal which is the largest of the protein-rich vegetable feed materials (oil seed meal) consumed.
2. Currently, data on the control of microbiological contamination of imported feed materials at source is limited.
3. *Salmonella* is the major hazard for microbial contamination of animal feed. *Listeria monocytogenes, Escherichia coli* O157 and *Clostridium* spp. are other hazards for which feed is regarded a far less important source. In addition, antimicrobial resistant bacteria, or antimicrobial resistance genes can be transmitted via feed.
4. Data of *Salmonella* contamination in forage is scarce, and in most studies non-processed cereals are reported to have a low prevalence of *Salmonella*, while available data demonstrates that non-processed soybeans are often contaminated with *Salmonella*.
5. This opinion focuses on industrial compound feed as the feed group with the highest risk for becoming contaminated by *Salmonella*. Oil seed meal and animal derived protein are the major risk feed materials for introducing *Salmonella* contamination to feed mills and industrial compound feed.
6. Several of the *Salmonella*-positive feed materials of both animal or plant origin, as well as industrial compound feed are produced in industrial processes (rendering and crushing plants and feed mills) where *Salmonella* should have been destroyed due to high temperatures employed in the production processes. This is caused by recontamination during cooling and further handling.
7. There is limited information on the occurrence of *Salmonella* associated with home-mixing of feeds.

8. Animals can become infected when fed with *Salmonella*-contaminated feed. This may occasionally cause clinical disease in some animals, but the major outcome is asymptomatic carriage. In addition, animals may also become infected from other *Salmonella*-infected animals, directly or via a contaminated environment for which the original source could have been contaminated feed. Transmission of *Salmonella* from animal feed to animals consuming the feed, and to food products derived from the animals has been shown.
9. The relative importance of different sources of *Salmonella* infections in animals varies. In regions with low prevalence status, where endemic infection is well controlled or absent, *Salmonella* contaminated feed is the major source for introducing *Salmonella* into the animal food production. In other regions with high prevalence, although it is difficult to quantify, the relative importance of feed as compared to other sources of *Salmonella* may be lower. In all situations, there is a possibility of introducing *Salmonella* in animal production via feed, which would compromise the results of other control measures.
10. Although the most common *Salmonella* serotypes occurring in humans are seldom found in animal feedstuffs in most countries, some serotypes found in feed are also found in humans.
11. There are safety benefits from the application of HACCP principles, GHP and GMP approaches in animal feed production.
12. Moist heat can effectively decontaminate feed materials, as well as compound feed as long as sufficiently high temperatures and treatment times are used. Where GHP/GMP are in place the risk of recontamination is minimised.
13. Comparative studies suggest that heat treatment processes used to successfully control *Salmonella* contamination will also be effective for other non-spore forming foodborne pathogens.
14. Although heat treatment is generally recognised as the most effective decontamination method, in some circumstances (e.g. pelleted feed for layers) this may not be appropriate. In such cases, chemical treatment of feed may offer an alternative means of protection.
15. Treatment of feed ingredients or compound feed with blends of organic acids, or with formaldehyde products (as a processing aid) at suitable concentrations, can be effective in reducing contamination by *Salmonella* and other organisms.
16. Chemical treatment has a residual protective effect in feed, which helps reduce recontamination. Also, the use of chemical treatments helps reduce contamination of milling and feeding equipment and the general environment.
17. The aim is for the feed manufacturer to continuously reduce the occurrence of *Salmonella* in feed for all food-production animals. Establishment of microbiological criteria for *Salmonella* contamination along the feed chain is appropriate and suggested below as one of several tools.
18. A feed safety criteria based only on testing of the end product would not be an effective way to ensure absence of *Salmonella* contamination. Establishment of one or more process hygiene criteria at critical stages of the feed production chain, including at the end product stage, is more efficient.
19. The importance of starting the control already at the crushing and the rendering plants is emphasised.
20. The currently applied sampling procedures can only reliably identify highly contaminated lots of feed materials and compound feed.
21. Culture according to procedure ISO 6579 is the standard method for isolation of *Salmonella* in feed. Alternative methods are used but not validated for detection in feed.

RECOMMENDATIONS

1. Comparable data on *Salmonella* in feed production at the EU level should be obtained, preferably by means of a base line survey (including information about prevalence in feed materials, compound feed and details of the production processes). These data could then be used to inform decisions to improve control of *Salmonella* in feed production.
2. More information should be gathered on the proportion of feed which is home-mixed for the various livestock species in EU MS, and to identify the sources of feed materials and procedures used by home-mixers, which may contribute to contamination with *Salmonella*.
3. Effective implementation of HACCP principles, and GMP/GHP procedures along the feed chain should be ensured. This requires proper control of recontamination, as well as determination of the effective heat treatments at the individual plants.
4. Common EU Process hygiene criteria should be established on crushing plants, rendering plants and feed mills as an integrated part of specific HACCP-based control programs to maximise the control of *Salmonella* contamination for all food-production animal species.
5. The ISO6579:2002 Annex D MSRV based method which has been adopted as the EU standard method for monitoring zoonotic *Salmonella* should urgently be validated for use in feed. Any alternative method should be equally validated for use in feed.
6. More research is needed on the relative efficiency of chemical feed decontaminants and their effect on subsequent *Salmonella* status of animals fed on treated rations. Also, a standard test model is required for chemicals used for decontamination of feed.

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APPENDIX A

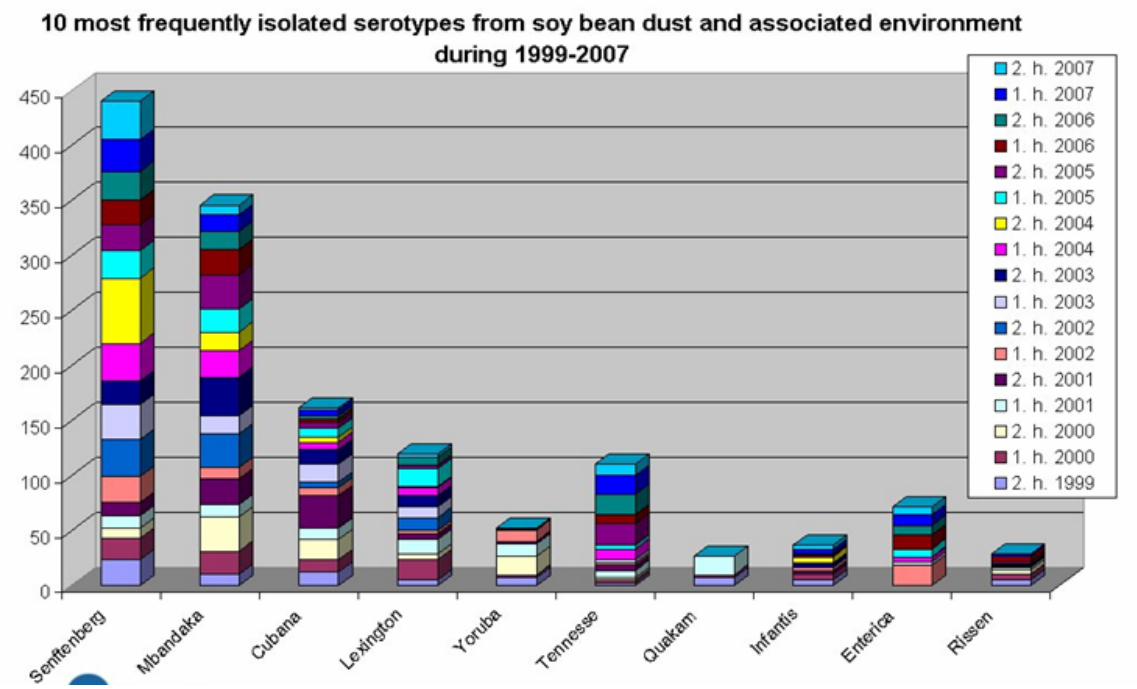

Figure I. **The ten most common serotypes of *Salmonella* isolated from soy beans imported from South America to Norway and from the environment of the importing crushing plant during the period 1999 – 2007. (Denofa, 2007) – data provided by industry.**

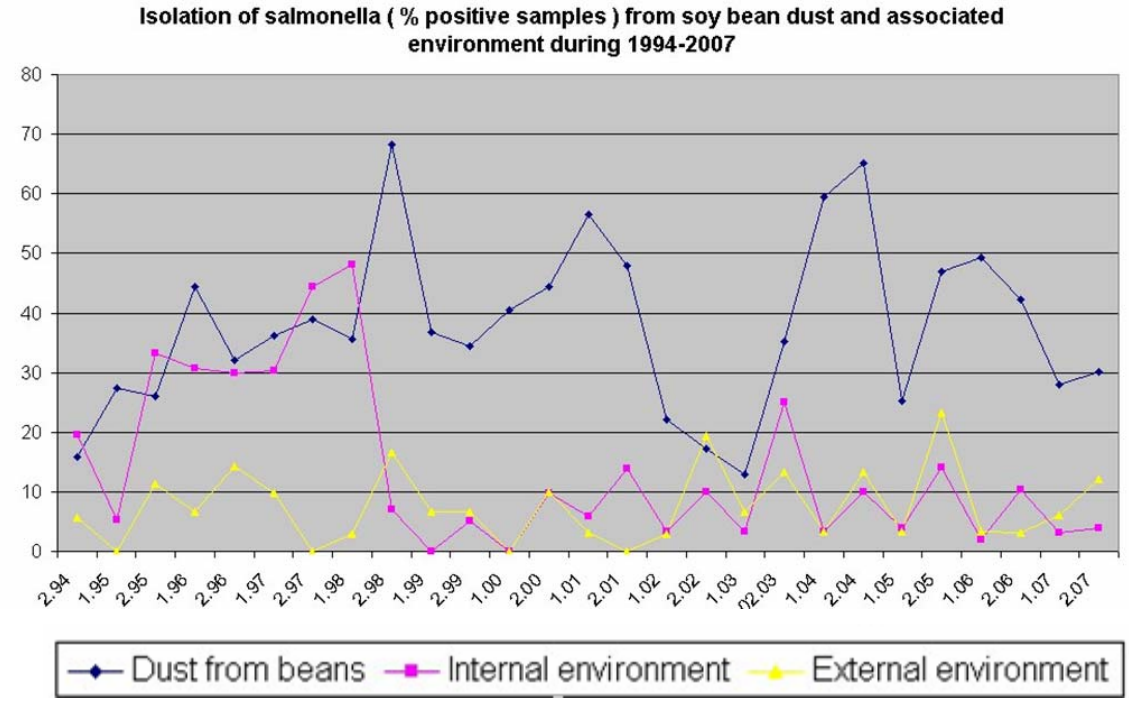

Figure II. **Isolation of *Salmonella* (% of samples) from samples taken from dust from soy beans upon importation from South America to Norway, as well as from samples from the internal and external environment of the importing crushing plant during the period 1994 – 2007. (Denofa, 2007) – data provided by industry.**

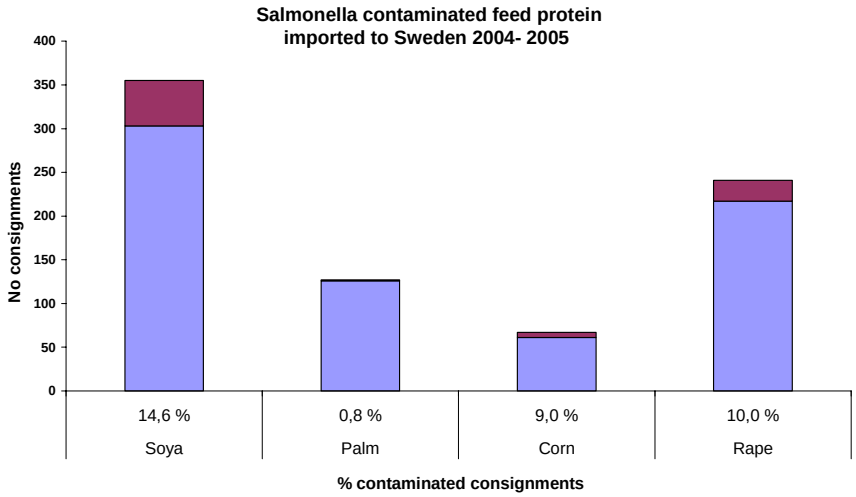

Figure III. **Total number consignments of different vegetable feed proteins imported to Sweden during 2004-05 and number found *Salmonella* contaminated (Wierup, 2006)**

Table I. **Import of soy beans and soy meal to the crushing industry in the EU-27 for the production of soy meal, oil and lecithin during Sept 2006 – August 2007. (Denofa, 2007) - data provided by industry**

Exporting country	**Soy beans** (1000 tonnes)	**Soy meal** (1000 tonnes)
Brazil	9.200	8.606
USA	3.550	78
Paraguay	870	-
Canada	630	4
Argentina	270	14.645
Norway	-	141
Other	330	33
Total	14.850	23.507

GLOSSARY / ABBREVIATIONS

Feed materials (often referred to as ingredients or straight feedingstuffs): various products of vegetable or animal origin, in their natural state, fresh or preserved, and products derived from the industrial processing thereof, and organic or inorganic substances, whether or not containing additives, which are intended for use in oral animal feeding either directly as such or after processing, in the preparation of compound feedingstuffs or as carriers of premixtures.

Compound feedingstuffs: mixtures of feed materials, whether or not containing additives, which are intended for oral animal feeding as complete or complementary feedingstuffs.

In annexes A and B of the Council Directive 96/25/EC (http://ec.europa.eu/food/food/animalnutrition/labelling/marktlab01_en.pdf) there is a glossary of the main processes used for the preparation of feed, as well as a list of the main feed materials.