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Microbial challenges of poultry meat production

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Food safety and shelf-life are both important microbial concerns in relation to broiler meat production. Focus is mainly placed on the absence or control of potentially pathogenic microbes such as Salmonella and Campylobacter but, from commercial point of view, other spoilage bacteria also play a role. Regarding food safety, the primary target should be the production of pathogen-free live animals, thereby allowing slaughter plants to keep the processing line free of those micro-organisms. Pathogen-free feed is fundamental in obtaining such conditions, as is the Good Hygienic Practice in farming, including grand parent stock (GPS), parent stock (PS) and hatcheries.

Interventions in the slaughter plant cannot always completely remove pathogens. However there are some measures of control available, including separation of flocks, carcass decontamination and implementing a balanced and operational HACCP system.

Shelf-life is closely linked to food safety during processing. The developments towards in-line processing, including chilling, portioning and deboning, allows optimal control. It minimizes processing time and product to product contact, and thus increases shelf-life and limits cross contamination. Refrigeration conditions are very important and an interruption of the refrigeration chain can accelerate microbial growth. Modified atmosphere packaging (MAP) may contribute in controlling the undesired growth of spoilage organisms, and can play a role in food safety as well.

The consumer needs to be educated in how to deal with food of animal origin that cannot be produced in an entirely sterile environment, in order to ensure shelf-life and correct preparation and use.

Keywords: poultry meat quality; food safety; Salmonella; Campylobacter

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Introduction

Microbial aspects of poultry meat can be divided into two major categories: food safety and spoilage. In the live phase of poultry production, food safety is the most important aspect, shelf-life is predisposed in the slaughter plant during processing of the meat, and during retail. In this paper a review is given on both aspects

Farm

Salmonella free poultry meat production starts on the farm, where biosecurity plays a major role in the control of infection and contamination. Every single part of the production system should be aware of its potential contribution in this respect. There are a wide variety of interventions and control measures available to limit infection opportunities.

At farm level, a number of interventions or preventive actions can be taken in order to control or eliminate food borne pathogens (Bolder, 2006b). These not only apply to growing broilers, but also to the preceding stages of poultry meat production and include hatcheries. At GPS and PS level vaccination against Salmonella, high standards of biosecurity on farm and regular monitoring in combination with other control measures are examples of such interventions. Broiler farmers should aim to start with pathogen-free premises and bought-in livestock should be pathogen free as well. It is now feasible to produce broilers without Salmonella, although Campylobacter-free production is still not possible, as even in so-called "high containment" facilities it appears to be difficult to keep Campylobacter out. The Swedish Salmonella control programme is often used as an example, and its strategy is to prevent, detect and eradicate Salmonella in animal production, including feed and processing of meat and eggs. No contaminated products are allowed to enter the market and infected flocks are killed and disposed of. From an economical point of view this approach can be very severe. Not much can be done to control the microbial load in poultry, except keeping the litter dry so carcasses stay visibly clean.

The design of poultry houses, equipment and also slaughter facilities should allow thorough cleaning and disinfection. The build-up of biofilm can easily occur when surfaces are rough and come into contact with animal effluent and secretions, especially protein and fat. Wooden surfaces are not easy to clean or disinfect, but in modern buildings wood is no longer used. The need for sanitation in poultry houses and hatcheries is obvious. As soon as a batch of infected animals leaves the farm, everything should be thoroughly cleaned out and disinfected. Verification of cleanliness should be performed before the next batch arrives. Sampling flocks of live birds can easily be done by the "overshoe" or "sock swab" method. The farmer can easily take the samples without much training and send the samples to the lab. There are different types of shoe covers, either plastic or a cotton-fibre material, and faecal material sticks better to the fibre material, although from our own experience plastic overshoes are useful in broiler house sampling. Skov *et al.* (1999) found that samples from five pairs of cotton fibre boots were as sensitive as samples from 300 individual broilers (pooled into 60 samples of 5 each), so sub-clinical flocks can also be detected.

Manure management needs attention in poultry production. In the Netherlands, manure has to be removed immediately after removal of confirmed Salmonella-contaminated animals to slaughter (Bolder, 2006b). In Sweden, manure cannot be applied on land where crops used for poultry feed are grown. Nicholson *et al.* (2005) found Salmonella could survive for up to one month in litter applied to arable land, and survive for up to 3 months in slurry.

Feed

Feed has to be safe, both from a chemical and bacteriological point of view. Feed components and raw materials can be vectors for transmission of zoonotic agents such as Salmonella. The majority of compound feed will be heat treated, so survival of pathogens is unlikely. The production line following processing may still provide a source of recontamination.

HACCP or GMP in the feed industry is supposed to offer a guarantee of the production of safe feed, but additional quality systems have been developed to compliment these schemes. TRUSQ in the Netherlands is such an example (Yntema, 2005). Practice shows that intensive monitoring and decontamination of raw materials is necessary. Lists of raw materials carrying a high Salmonella risk exist in several countries *e.g.* Sweden, and include fish meal and rapeseed meal, and soy bean meal (Häggblom, 2006). Not only feed ingredients or finished feed, but also the entire feed processing line can be at risk (Davies and Wray, 1997). Salmonella infection through feed is often underestimated, because one of the difficulties is the collection of representative samples both from raw materials and finished feed (Häggblom, 2006). Feed can be a vector in the Salmonella epidemiology in broilers, although, in The Netherlands, Salmonella serotypes isolated from feed remain to be identified in broilers.

Important risk factors in the feed mill include: 1. the Salmonella contamination of raw materials, 2. insufficient heat treatment, 3.condensation in the coolers after pelletising or expanding, 4. application of inadequate cleaning and disinfection programmes.

On the farm, feed contamination points include: 1. silo management (Bolder, 2006b), where condensation inside the silo needs to be monitored, 2. auger systems from the silo into the house, 3. auger systems inside the house to the feeder pans, which often start at an open small feed bin, 4. application of whole grain added to the feed.

Acidification of feed can help reduce contamination, but is no guarantee for preventing transmission to poultry. Application of such interventions for raw materials can mask the risk, and lead to less stringent hygiene practices and disease monitoring.

Conditioning and pasteurization should be applied to all types of animal feed, including high risk raw materials. Intensive monitoring of the production line keeps the quality manager regularly informed about the Salmonella status in the feed mill.

Live bird handling

From welfare point of view live bird handling has improved considerably over the past years. Small coops have been replaced by large containers, with substantially bigger loading openings. Mechanical harvesting and unloading of containers had further improved welfare. Effects of feed withdrawal are primarily negative from a contamination point of view.

Live bird handling causes stress in the animals, which leads to excessive faecal excretion. Recently a number of studies were performed providing information on the background of stress. Automated harvesting may not only reduce the physical damage to birds, but can also be a less stressful process. Gregory (1996) reviewed pre-slaughter handling systems, and referred to the studies of Rigby and Pettit (1980), who found an increasing number of birds shedding Salmonella during transport that lasted for a maximum of 4 hours. Longer transportation times lead to a lower prevalence. Brown *et al.* (1995) showed that reduction of stress was obtained by changing transport conditions: for instance the switch from small coops towards larger containers. Heat stress increases faeces production, so it is important that the animals can regulate their body temperature, and that no thermal imbalances are introduced.

Broilers are typically kept without feed for a few hours before loading. Feed deprivation is important in relation with the faecal shedding during transport and determines the amount of faeces present during slaughter. Zuidhof *et al.* (2004) reported an optimum feed withdrawal time of 12 hours in relation to carcass contamination, without loss of bird weight. Warriss *et al.* (2004) noticed increasing fluid contents in the gut after feed withdrawal, which may lead to more cross contamination especially in the case of slaughter failure. The crop is increasingly considered an important source of contamination. During prolonged feed withdrawal periods, the *Salmonella* load in the crop increases. Broilers may start litter-pecking, consuming feed remnants, faeces and small feathers. Moreover, there is a change in crop environment, whereby it becomes less acidic and more attractive to pathogens like *Salmonella* and *Campylobacter* (Northcut, 2001). Liquid crop contents, although difficult to observe, have been reported to contribute to increased numbers of *E. coli* and *Campylobacter* on carcasses (Smith, 2005a).

Feed withdrawal affects the amount and the condition (watery or firm) of the digesta as well as the integrity of the gastro-intestinal tract. Watery gut contents can leak out more easily, posing a contamination risk during processing. In case of decreased gut integrity, there is a higher chance of gut damage and spillage of digesta during evisceration. The Campylobacter CFU counts of freshly produced faecal material in transportation containers at arrival in the slaughter plant are higher than in fresh faecal samples in the broiler house before feed withdrawal (Nauta *et al.*, 2007). This can be explained by the presence of relatively higher proportions of caecal material. Byrd *et al.* (2003) significantly reduced Salmonella in the gut of broilers by administering a commercial chlorate product during the 10 h feed withdrawal period, although they did not monitor the effects during transportation or in the processing plant. The question is whether the broilers may actually be more exposed to contamination as a consequence of excessive shedding of Salmonella during transported. Jacobs *et al.* (1999) found no effect of feed withdrawal on the Campylobacter contamination of crates or broiler carcasses after short transportation periods.

Slaughter plant

At the slaughter plant, contamination is restricted to zoonotic agents. The general microflora, including the part which determines shelf-life, is also very important. Ultimately, hygienic conditions at the slaughterhouse have to be maintained in order to create conditions where the microflora of slaughtered animals can be controlled. Certain bacteria often are used as indicators of the presence of pathogens from faecal origin. Bacteria that are responsible for spoilage of poultry meat products, including those from faecal origin, present only a relatively small part of the total count, approx 10%. Other spoilage vectors were present either on the live animal or were introduced during slaughter and further processing. There is little that can be done to decrease the total bacterial count on live animals, except trying to keep the birds dry during transportation. Humid conditions contribute to spread and growth of bacteria on the skin. Here faecal bacteria do play an important role. Clean feathers reduce the bacterial load during the first processing steps; unloading, killing, scalding and plucking.

Microbial food safety should not only be controlled by HACCP at the slaughter house. Contamination should be controlled at farm level, followed by pre-screening and physical separation of contaminated flocks. Of course this system needs a consistent and reliable monitoring of flocks, particularly at the end of the grow-out period. However for microbes such as Campylobacter, with high prevalence during summer, separation is difficult to justify on an economical basis.

In slaughter houses carcasses are transported via conveyors where they may be contaminated by aerosols or condensation formed on the equipment or ceiling. This water will be heavily contaminated and can influence shelf-life of the final products. The development of so called "house flora" which hides in unexpected corners and can contaminate subsequent batches is particularly hazardous (Bolder, unpublished data). Guided air flow from clean to dirty parts of the processing area may help to avoid condensation formation. Under normal conditions, the microflora varies at the different stages of the process. Modern slaughter lines are highly automated without much manual labour. 'Cleaning In Place' (CIP) systems are installed on modern equipment, but are only applied during breaks or at the end of processing shifts. This should be available for all types of machines, which are mostly carrousel shaped and have to perform complicated operations in line. There should be no "hidden" areas where sanitation is impossible. Of course the buildings also have to be "food production proof".

Developments of recent years are listed in *Table 1*. They have a potential impact on the food safety or bacteriological quality of poultry carcasses.

Heemskerk (2005) reviewed the recent literature on the slaughter process and came to the conclusion that improvements on the hygienic situation could only be obtained by intervention at several places in the slaughter process at the same time, a so called 'hurdle' approach. He suggested that all major infection routes should be considered at the same time. Defecation during the early stage of the slaughter process appears to contribute in this respect. Hinton *et al.* (2004) saw cross contamination in all processing steps, and even on successive days the same bacteria could be found. Thorough cleaning and disinfection of the slaughter plant and equipment is a professional business, which has to be taken extremely serious (Loughney, 2004).

In the USA attention has been focussed on post-harvest food safety (Russell, 2003). Salmonella control measures in live animals seem to be behind schedule, although plans are in place to apply this approach to the whole poultry production chain (Russell, 2002). Government control actions mainly focus on the control in processing, with special attention given to water chilling and reprocessing of carcasses. Carcass decontamination

Table 1 Improvements in poultry processing plants over the past 15 years.

1.	Containers have replaced small plastic coops
2.	Gas stunning is becoming more popular, with less infection of the air sac, less physical damage to the birds and less defecation while being stunned
3.	Counter current and multi-stage scalding systems, where carcasses are washed during scalding
4.	Pluckers can be easily turned inside out, allowing cleaning during breaks and more efficient cleaning and disinfection after production, so more efficient hygiene is achievable
5.	Re-hanging is fully automated, so cross-contamination during the piling up of carcasses no longer occurs
6.	'Cleaning In Place' is installed on modern equipment
7.	Modern equipment can be easily adjusted, avoiding damage to intestines at opening machines and during evisceration
8.	During carcass opening, a vacuum system removes rectal contents and the cloaca is positioned at the back of the carcass, avoiding contamination of the carcass with intestinal contents
9.	At evisceration, intestines are physically separated from the carcasses for inspection
10.	Final washing and inspection of carcasses is fully automated and reliable, so no human checks are necessary
11.	Air chilling
12.	Introduction and application of HACCP
13.	Introduction of automatic portioning lines
14.	Vision systems introduced
15.	Electro-stimulation or maturation at different stages of the process allowing in line processing including chilling, portioning and Deboning
16.	In-line processing with minimal contact between carcasses and with improved tracking and tracing

is an important step here, evidenced by the large number of papers on this subject. HACCP in poultry processing is gaining attention, and soon after introduction of this system in the US, there was an obvious fall in Salmonella contamination of poultry products. Unfortunately HACCP in poultry processing alone is not enough to maintain this downward tendency. As stated previously, there is a clear need to control pathogens in live birds that are ready for slaughter.

Containers have replaced small plastic coops, but, like coops, containers are not free of pathogens after cleaning and disinfection. Transmission of Salmonella or Campylobacter may therefore happen from one flock to the next (Slader *et al.*, 2002; Hanson *et al.*, 2005). In a longitudinal study with broilers, Corry *et al.* (2002) showed that transportation crates or containers may not be free of Salmonella after cleaning and disinfection. Cleaning and disinfection of transport containers is only possible when a multi-stage cleaning system is applied: 1. soaking, 2. flushing with large amounts of water to remove organic materials, 3. washing stage using detergent and the right temperature under high pressure with correctly adjusted nozzles that can reach all parts, inside and out 4. flushing off detergent residue and removal of excess water with high pressure air, 5. disinfection. Containers have to be perfectly clean in order for disinfection to be effective.

Automated unloading of containers followed by transportation of live birds into the hanging area, whether via a gas-stunning tunnel or not, poses microbiological challenges when cleaning these complex and covered systems. Gas-stunning is becoming more popular and may prevent infection of the air sac and causes less physical damage to the birds in comparison with water-bath stunning. A gas stunner is relatively uncomplicated and should be easily cleaned, and accessibility of all carcass contact areas is essential. This also applies to other equipment for live bird transportation which is not exposed to daylight.

Electro-stimulation (ES) of carcasses can be carried out either during bleeding or after plucking. In both cases hygiene problems may occur. When ES is applied during bleeding, faeces may be expelled by convulsions and contractions. After plucking, there are possibilities for cross-contamination from a relatively heavily contaminated area via the contact plate.

Many modern processing plants now have counter-current and multi-stage scalding systems, where carcasses are washed during scalding. Together with the switch from immersion-chilling to air-chilling, the scalding temperature has changed from approx 60°C to 52-54°C, which has hardly any lethal effect on bacteria (Mulder and Dorresteyn, 1977). Cason *et al.* (2000) experimented with a three stage counter-current scalder and found that the majority of the microbial flora was washed off during the initial process. Kim *et al.* (1993) saw no differences in microbial CFU counts between scalding temperatures of 52°C, 56°C and 60°C, although SEM pictures showed differences. Soft-scalded skin (including epidermis) appeared to have fewer bacteria attached. Changes in the pH of scald water can reduce D-values of micro-organisms in the scald water (Humphrey *et al.*, 1984; Bolder, 1998).

During breaks in processing, it is obvious that the bacterial counts of scald water decrease, but soon after resumption, contamination levels will be restored to the previous high level. Some processing plants heat up the scalding tank after cleaning and refilling above 75°C and let it cool down overnight until the next shift. Such practices result in water free of coliform bacteria, and no Salmonella or Campylobacter can be found (Bolder 2003, unpublished results).

Pluckers can be turned inside out during cleaning, allowing more efficient hygiene practices. Plucker fingers can easily become permanently contaminated, especially in any hair-line cracks. Frequent replacement is therefore necessary, which helps control the spread of pathogens and spoilage organisms. Washing pluckers during and after use is not

only important to remove feathers, but also delays bacteria from attaching to the processed carcasses. Regular rinsing of carcasses will help to inhibit attachment of microbes, especially those that exhibit freshly produced faecal material during the plucking process. In relation to pathogens, many may already be present on the skin in the poultry houses or during transportation. Bacteria can either be attached reversibly or permanently. The close contact of plucker fingers with the carcasses may rub-in organic material and micro-organisms. Application of water in the pluckers can be considered as a processing aid, as feathers are easier removed from the pluckers and water provides lubrication. Air in the plucking area can be highly contaminated (Berrang *et al.*, 2004). Rehanging can be fully automated, so cross-contamination during piling up of carcasses no longer occurs and no manual handling of carcasses is necessary.

Modern slaughter equipment can be easily adjusted, which helps avoiding damage to intestines at opening machines and during evisceration. Here the intestines are separated physically from the carcasses for inspection, which prevents contamination (Mulder and Schlund, 1999). The removal of an intact intestinal package is very important in order to prevent the spread of faecal material and bacteria on the carcasses. Correct adjustment of the carcass opening machines is crucial in this respect, and homogeneity of flocks contributes to the success of this step in processing. Final washing and inspection of carcasses is now fully automated and more reliable, so human quality checks are seldom necessary at the end of the evisceration line.

Vision systems offer the possibility to identify the unbled, damaged and soiled carcasses and can be used for pre-selection before veterinarian checks. Those carcasses can then be excluded from the normal process or be reprocessed, although the veterinary inspection should have picked them out. Vision systems however can also be used to check carcass quality *e.g.* before cutting up, or elsewhere during the process.

Chilling with air is becoming more popular worldwide, although studies on the bacteriology of air chilling do not show any reductions in pathogen or bacterial counts (Allen *et al.*, 2000; Flucky *et al.*, 2003). In a so-called 'vent stream' chiller the number of Pseudomonas may be reduced, but application of spraying during air-chilling had an adverse effect on the same CFU counts. Mead *et al.* (2000) reported the spread of a marker organism in the air-chiller in all directions. An alternative solution for using water spray in air-chilling systems might be provided by including a pre-chilling step by immersion before air-chilling (Logtenberg and Stekelenburg, unpublished data).

During immersion chilling in water an equilibration of contamination occurs, not only by the spread of pathogens from contaminated to uncontaminated carcasses, but also in increased uniformity of CFU counts after the chiller, in comparison with air-chilled carcasses (Smith *et al.*, 2005).

Carcass decontamination is widely used in immersion chilling operations, but appears to be ineffective when spray application and air-chilling has to be applied (Bolder, 2003; Bolder, 2006a), as contact time during spraying appears to be too short. In the EU, chemical carcass decontamination is not (yet) allowed. At the time of writing, EFSA (European Food Safety Agency) is preparing a list of chemicals that can be used.

The shelf-life of poultry products can be influenced by a number of processing conditions. Initial bacterial counts dictate the period required for the microflora to increase to undesirable levels. Temperature during storage is important, as is the type of microflora that is present on the meat. As the product reaches and exceeds its shelf-life, the presence of high CFU counts (10 million CFU micro-organisms per gram), particularly of Pseudomonas, can cause slime and rancid odours.

The type of slaughter process plays an important role. High scalding will result in loss of bird epidermis and causes differences in growth conditions for the microflora, especially in case of water chilled, non-frozen storage. Low scalding and dry-chilling

leads to dry skin with different attachment and growth conditions for microbes. However, in modern poultry processing, most of the products on the market are further processed, often without skin, which creates comparable conditions for both types of products.

The lower the initial counts, the longer the shelf-life of the final meat products. Of course bacteria that can grow at low temperatures (psychrophiles) remain important. An uninterrupted refrigeration chain is key to controlling the growth of spoilage bacteria. Condensation on meat or skin surfaces creates a favourable environment for the development of bacteria, however the a_w of these surfaces must be optimal for bacteria in the absence of condensation. Storage temperature remains important in this respect (Hinton, 2000).

Poultry meat products can either be packed on trays wrapped either in plastic foil or with a sealed cover. Packing of meat under a modified gas atmosphere (MAP) is not common for several reasons, however shelf-life and food safety can be both improved by employing this technique (Boysen *et al.*, 2007; Church *et al.*, 1995 and Nychas *et al.*, 1996). Poultry meat has a quick turnover time in retail marketing, and the extra costs for packing materials and equipment might be a problem. Both from an economical and public health point of view, the interest from retailers is increasing and fresh poultry meat products under gas can now be found in shops. Consumers however have to be informed that gas packaging does not replace refrigeration.

Further processing and retail

Plants for portioning or cutting up and further processing of poultry meat products should also apply GHP conditions, which can be based on a HACCP plan. This type of plant normally receive pre-cut broiler parts, which may come from several slaughter houses, making tracking, tracing and quality control of raw materials difficult. Further processing of poultry meat has its own specific hazards. Brine injection of breast meat, for example, is a hazardous operation, because it may introduce intramuscular contamination (Gill *et al.*, 2004). Similar problems do exist at seasoning, tumbling, grinding and reshaping. Deboning operations for breast meat have existed for many years, but leg or thigh deboning is now also available. For this type of equipment cross-contamination is simply not possibility.

Both retailers and consumers have to be made aware of the potential dangers that exist in fresh food from animal origin. In the US, FSIS gives information via a web site regarding how to handle food, *e.g.* for barbeques, which includes the sale, transportation to the home, defrosting, marinating etc. The importance of kitchen hygiene in this context is stressed.

Heat treatment or pasteurisation of meat products increases food safety, however so called “pre-cooked” products composed of minced meat may not be pathogen free. Besides that, recontamination during chilling and packaging may also occur. Undercooking of these types of products can cause gastric infections. Consumers should also be aware that frozen meat products are not always free of pathogens. Increasing the food safety level is expensive and not without risks (Rougoor *et al.*, 2003) However it must also be recognised that without regular challenge, the immune system of both man and animals will not be sufficiently prepared. Minor challenges can cause undesired or very severe reactions.

Conclusions

Food safety aspects of poultry meat production should be mainly controlled at farm level, so that, during the slaughter process, uncontaminated conditions can be maintained.

There are working programmes available for the control of Salmonella in broiler farms, based on the so called 'hurdle' principle. Single interventions have proven insufficient for eradication or control of carcass contamination. Unfortunately Campylobacter control in poultry is still not possible, and the focus remains on the slaughter process.

When uncontaminated live animals arrive at slaughter, there is a good possibility they will remain so if handled at a modern processing plant. Unfortunately this demands a reliable pre-screening of flocks and strict separation. Without these measures, cross-contamination will occur. GHP can support the production of bacteriologically stable meat products with long shelf-lives. On the farm, carcass contamination can be influenced by maintaining good litter quality.

Treatment of slaughtered carcasses or meat products can be the only way to remove contaminants. Carcass decontamination and MAP packaging may be effective interventions here.

Whilst no guarantees can be given for the production of pathogen free products, consumer education continues to play an important role in protection against gastric infection.

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