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# An evaluation of microbiological intervention techniques within broiler production

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# An Evaluation of Microbiological Intervention Techniques within Broiler Production

A thesis submitted for the degree of Doctor of Philosophy to Bangor University

by

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## Thesis Abstract


Meat hygiene is a key measure of both food safety and quality, intrinsic in today's competitive retail market. Profit margins in the poultry-meat sector in particular are very narrow with producers constantly looking at improvements throughout the "plough-to-plate" production process. However it is paramount that any improvements are safe to consumers and to the wider environment.

*Campylobacter* Spp. are the largest cause of bacterial food-borne gastro-intestinal disease in the western world and are a major focus of research by government authorities such as the UK's Food Standards Agency, as strong links have been made between the incidence of *Campylobacter* in broilers and the disease in humans. The infectious dose for *Campylobacter* spp. in humans is very low. This means that high standards of hygiene are critical at all levels of the broiler production process. Broiler industry workers were surveyed as to their knowledge of the issues surrounding *Campylobacter* spp. and found little evidence of specific knowledge regarding the bacteria, though did find evidence that general food safety measures are widely known. The results of this survey could be critical in designing education programs for workers, enabling knowledge-gaps to be addressed.

Blood waste disposal from poultry abattoirs is a considerable cost to producers as legislation states that it must be sent for heat treatment (often over long distances). However, one chapter within this thesis shows that lime treatments (both Calcium oxide and calcium hydroxide) are highly effective in killing inoculated ($10^9$ CFU) pathogenic bacteria (*E. coli* O157:H7 & *Salmonella poona*), even at low levels of lime (2.5%). This mixture has also been shown to give agricultural benefit to perennial ryegrass (*Lolium perenne*), when added at typical commercial application rates. Thus, lime treatment is a safe and beneficial alternative disposal method for blood waste, which therefore has the potential to cut costs for both broiler producers and local farmers.

Damaged chicken wings are currently disposed of as category-3 hazardous waste due to Meat Hygiene Service legislation. However, the study contained in this thesis shows there to be no difference in either pathogenic or spoilage bacterial loads between normal wings and damaged wings. Longitudinal studies have investigated this further and have found that the vast majority of physical damage occurs post-mortem rather than pre-mortem, which minimises the opportunities for the ingress of bacteria into the broken wing.

Different production systems have also been examined for the incidence of broken wings, with RSPCA freedom foods birds having significantly less breakages (both pre- and post-mortem) than equivalent sized (2.2kg) conventional birds. Heavier conventional birds (3.8kg) were also shown to have a greater incidence of broken wings than standard conventional birds (2.2.kg).

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# Chapter 1

## Introduction and Literature Review

# 1 Introduction and literature review

## 1.1 Thesis introduction

Poultry meat is an intrinsic part of global human nutrition. It is the second most consumed meat in the world, and most consumed meat in the UK (44% of all meat). Of UK poultry meat, 85% is from broiler chicken production, arising from slaughtering 850 million broilers in the UK per year (FAOSTAT, 2009).

Not only is chicken meat a source of protein for humans, but it is also low in fat and poses few social taboos around its consumption. Additionally, the mass industrialisation of the broiler industry that has occurred since the 1950s (Kettlewell and Turner, 1985; Delezie et al., 2006) combined with advances in genetics, breeding, management and nutrition have lead to a significant reduction in time taken to produce the standard 2.2 kg bird for slaughter. As a direct result of such reductions in the cost of production and low profit margins, poultry meat has become more affordable for consumers, and together with being a low-fat source of protein, has become the most consumed meat in the UK.

However, this has come at a cost. In the drive for ever more competitive products and higher yields, some factors have been overlooked. It has been well documented how historically welfare has been compromised, and out of public perception have come assurance schemes such as the RSPCA freedom foods standard.

Furthermore, it is essential that the food available to consumer is safe for them to eat. Figures show that whilst human cases of *Salmonella* spp. are falling, others such as *Campylobacter* spp. have been increasing in the last few years (FSA, 2009). Food-borne diseases in England and Wales are estimated to cost the economy £1.5bn per year (FSA, 2008) in terms of hospital care, lost productivity and a notional cost of the freedom from disease, and thus any improvement in general food hygiene would lead to significant cost savings to the economy (FSA, 2008).

Poultry has been well documented to harbour a number of pathogenic bacteria (Hwang and Beuchat, 1995) such as *Campylobacter* (Moran et al., 2009), *Salmonella*

(Dominguez et al., 2002; Harrison et al., 2001), *Listeria* (Pini and Gilbert, 1988), and *E. coli* O157:H7 (Akkaya et al., 2006).

In addition to the human health implications of disease outbreak, there is a commercial aspect too, as sales typically plummet in the aftermath of the negative media coverage associated with an outbreak as a number of public-heath scares have shown. The BSE crisis reduced the sales of beef in the UK from 617,000 tonnes in 1988 to 390,000 t in 1996 (Yeung and Morris, 2001). Similarly, public concerns about *Salmonella* spp. in eggs reduced the UK consumption from 8,270 million eggs in 1988 to 6,556 million in 1989 (Yeung and Morris, 2001). Thus food safety is not only vital for the consumer's health, but also important for commercial companies who cannot afford such sudden drops in demand for their products.

This is particularly true in the broiler industry where tight profit margins are constantly under pressure (Sheppard, 2004). Although the industry is on a large scale and thus has a high financial turnover, the profit margin per bird is very low (Cevger and Yalcin, 2003), reported to be 1.9p/bird in 2005 (Hughes et al., 2008). Thus there are financial pressures on companies as well as legal and moral obligations to ensure their products pose as little risk to consumers as possible.

Chicken wings are one of the carcass cuts most likely to harbour pathogenic bacteria (Uyttendaele et al., 1997). Studies have found high prevalences of pathogenic bacteria species such as *Campylobacter* (FSA, 2009), *Salmonella* (Capita et al., 2003), *Listeria* (Uyttendaele et al., 1997). Current meat hygiene regulations (Anon, 2007) state that damaged wings, with bones protruding through the skin, must be disposed of as category-3 hazardous waste, which is considered not fit for human consumption. However, it seems that no scientific research has been conducted upon the risks damaged wings pose to consumers. Obviously in the climate of minimal profit margins, any potential savings in disposal costs, coupled with potential income from sales of damaged wings could aid poultry companies and secure jobs.

Similarly, other poultry abattoir wastes add disposal costs. Blood is another category-3 waste, and under current regulations must be either rendered or incinerated (Anon, 2005). However, these facilities are often a considerable distance from abattoirs. Thus processors incur not only disposal charges and transportation costs, but also this process

is responsible for $CO_2$ emissions, which are currently being critically assessed by supermarkets with a view to minimising their emission levels. Blood waste disposal is not only an economic constraint on the industry impinging on tight profit margins, but is also a loss of valuable nutrients for local farmers.

Finally, the incidence of *Campylobacter* Spp. in humans is currently the number one food hygiene concern in the European Union. A great deal of research has and is being conducted to reduce the incidence of human disease. Indeed, the European Food Standards Agency (EFSA) has a target of reducing the incidence in humans by 50% by 2010. Their main focus of research in order to achieve this has been the incidence of *Campylobacter* spp. in poultry meat, as this is linked to approximately 40% of human *Campylobacter* cases. To this end, the UK's FSA has set the target of a 50% reduction in the incidence UK produced chicken meat by 2010 (FSA, 2008), though notably this does not include imported chicken meat. As the level of *Campylobacter* Spp. decreases during processing (the bacteria only reproduces in the gastro-intestinal tract of animals), research is required on methods of reducing the level and prevalence in live birds and practices to limit contamination during processing. Together, these would plausibly give producers some measure of control over the bacterial levels in the chicken meat they produce.

Thus, food hygiene determines health and quality of the product to consumers. Waste hygiene is equally important to the health of the population too. When combined they have the potential to reduce the production costs whilst decreasing risks to the general public.

### 1.1.1 PhD Thesis Aims

The aims of this PhD are to investigate a number of areas within chicken production, predominantly from a microbiology perspective, though also considering other factors such as animal welfare. These are noted below:

#### 1.1.1.1 Determine the knowledge level and identify knowledge gaps surrounding *Campylobacter* spp. in broiler industry workers.

It is a generally held belief within the UK broiler industry that the workers employed in various aspects of broiler production have little knowledge about *Campylobacter* spp.. Other diseases such as Avian influenza and *Salmonella* spp. receive widespread media

coverage and are subject to food safety training programmes, whereas *Campylobacter* spp. does not.

Therefore by conducting questionnaires on broiler industry workers their knowledge level regarding *Campylobacter* spp. can be assessed. Furthermore, using these responses, trends in the absence of specific knowledge can be detected. From this it will be possible to see whether these the absence of knowledge potentially gives poor practices which promote conditions that encourage the survival and transfer of *Campylobacter* spp. throughout broiler production.

#### 1.1.1.2 Devise an alternative procedure to safely dispose of poultry blood waste.

Currently poultry blood waste is typically transported over long distances for processing prior to safe disposal or usage. However, this deprives farmers in the vicinity of poultry abattoirs a valuable local source of nutrients for application to their land. A treatment that could be used in the locality would help redress this issue. The research in this thesis therefore aims to investigate whether poultry blood waste, inoculated with pathogenic bacteria can be successfully sanitised using different types and levels of lime.

#### 1.1.1.3 Investigate whether damaged chicken wings are fit for human consumption.

As mentioned previously, current meat hygiene regulations prevent the sale of damaged wings for human consumption. The research in this thesis aims to compare the level of bacterial contamination on damaged and undamaged wings and to determine whether damaged wings have greater food safety risks associated with them.

#### 1.1.1.4 Investigate how and at which stages of production wings are actually being damaged, and whether the incidence of damage varies between production systems.

This thesis aims to take the research on damaged chicken wings a stage further than simply the food safety. By sampling the incidence of wing damage at various stages of production the damage can be attributed to certain practices or processes. Furthermore the research aims to compare different production systems (conventional and the RSPCA's freedom-foods standards) to see whether the incidence of wing damage varies between systems.

The thesis begins with a review of the relevant literature on these topics.

## 1.2 Overview of Poultry Production

Chicken meat is produced through a variety of methods and throughout the world. There are few major religions or social taboos that prohibit the consumption of chicken meat (FAO, 2006) and it is the second most eaten meat in the world (FAOSTAT, 2009). As Table 1.1 and Figure 1.1 below show, this trend is rising and the growth of chicken meat production is greater than for any other category of meat. Whilst the production of chicken in 1990 was at a level equivalent to only of half pork production, this has risen to just over two thirds. It is not inconceivable therefore that in the fullness of time chicken will overtake pig-meat as the world's most consumed meat.

**Table 1.1** – World meat production

| | 1990 | 1997 | | 2007 | |
|---|---|---|---|---|---|
| Type of Meat | Production ('000 t) | Production ('000 t) | Growth in production from 1990 (%) | Production ('000 t) | Growth in production from 1990 (%) |
| Meat, total | 180,000 | 216,000 | 20.1 | 265,000 | 47.4 |
| Pig meat | 70,000 | 83,000 | 18.9 | 103,000 | 46.7 |
| Beef and buffalo meat | 56,000 | 58,000 | 4.8 | 64,000 | 14.3 |
| Sheep and goat meat | 10,000 | 10,000 | 8.2 | 13,000 | 34.3 |
| Poultry meat | 41,000 | 60,000 | 45.7 | 81,000 | 97.4 |
| Chicken meat | 35,000 | 51,000 | 43.5 | 70,000 | 97.3 |
| | 1990 | 1997 | | 2007 | |
| Type of Meat | Market share (%) | Market share (%) | Market share growth from 1990 (%) | Market share (%) | Market share growth from 1990 (%) |
| Pig as % of total | 38.8 | 38.5 | -0.4 | 38.7 | -0.1 |
| Chicken as % of total | 19.7 | 23.5 | 3.8 | 26.4 | 6.7 |
| Chicken to pig meat | 50.8 | 61.2 | 10.5 | 68.3 | 17.5 |

Source data: FAOSTAT (2010)

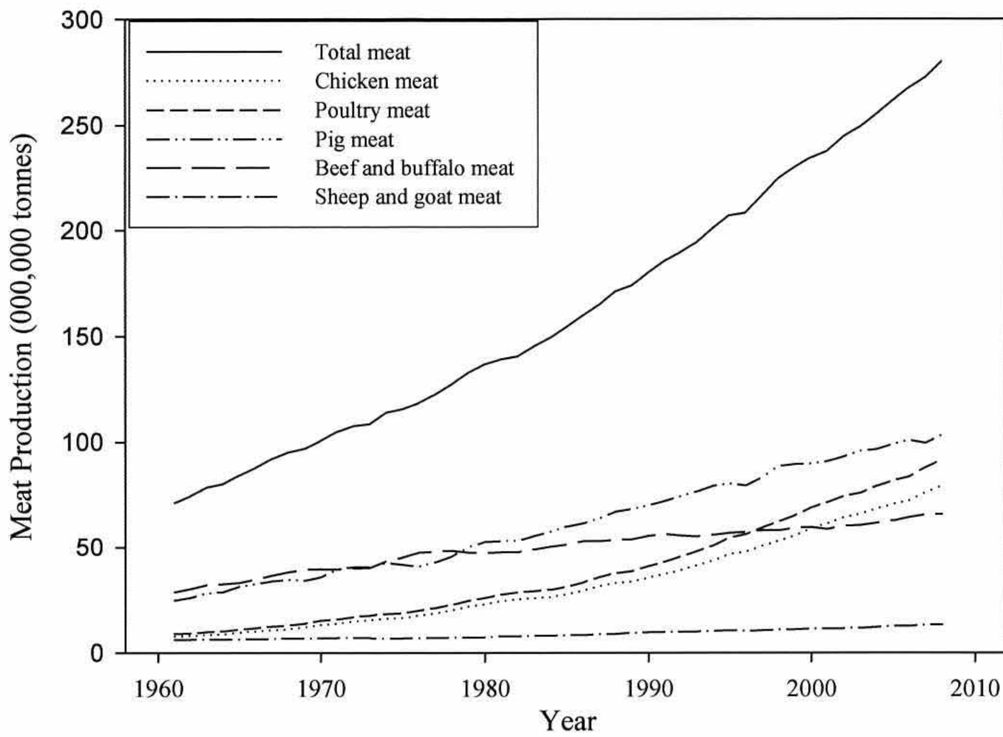


**Figure 1.1** – Trends in world meat production

Source data: FAOSTAT (2010)

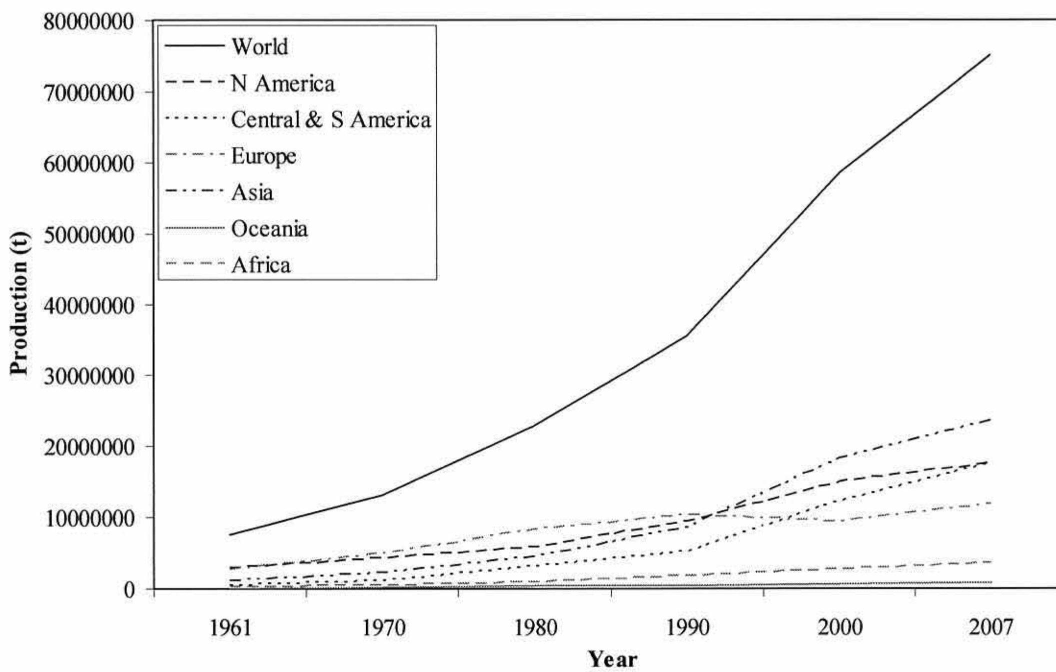


**Figure 1.2** – Trends in world chicken meat production

Source data: FAOSTAT (2010)

As figure 1.2 shows, the world production of chicken meat has dramatically increased since the 1960s. This has been due to the mass industrialisation of broiler meat production in many areas of the world. Initially this was in the western countries of Northern America and Europe, although in more recent times there has been rapid growth in Asian, South and Central American countries (FAOSTAT, 2010). This growth in chicken meat production has followed these countries' rise in economic strength and the resultant increase in their standards of living. A secondary driver for this has been the growth of chicken production in these newly industrialised countries (e.g. Thailand and Brazil) for export to western ones, helped by the cheaper labour costs and close proximity to feed raw-materials (e.g. Soya).

**Table 1.2** – World production and consumption of chicken meat

| Area | Chicken meat production, 2005 | | | Chicken meat production 2003 (tonnes) | Population (Est. 2003) | Consumption per capita (kg/year) |
|---|---|---|---|---|---|---|
| | Slaughtered birds (000s) | Production (tonnes) | Ave meat yield (kg/bird) | | | |
| World | 48132160 | 70,008,289 | 1.45 | 65,990,290 | 6,301,463,000 | 10.472 |
| UK | 848000 | 1,309,000 | 1.54 | 1,294,783 | 59,470,000 | 21.772 |
| USA | 9150000 | 16,025,900 | 1.75 | 14,924,000 | 294,043,000 | 50.754 |
| Africa | 2795407 | 3,240,120 | 1.16 | 3,188,369 | 850,557,000 | 3.749 |
| Asia | 17991780 | 22,308,842 | 1.24 | 21,349,826 | 3,823,389,000 | 5.584 |
| Europe | 7581737 | 10,836,412 | 1.43 | 10,284,448 | 726,339,000 | 14.159 |
| Latin America and Caribbean | 9434893 | 15,701,991 | 1.66 | 14,442,453 | 543,246,000 | 26.585 |
| N America | 9795086 | 17,026,003 | 1.74 | 15,877,606 | 325,698,000 | 48.749 |
| Oceania | 533257 | 894,921 | 1.68 | 847,589 | 32,234,000 | 26.295 |

Source Data: FAOSTAT (2009)

As Table 1.2 shows, although Asia is the greatest producer of chicken, consumption per capita is greatest in the USA and the lowest in Africa.

Throughout the world, chicken has the highest feed conversion ratio (FCR) of any domesticated farm animal (FAO, 2006). Typically, in intensive broiler systems in the Western world, target weights are 2.2 kg live weight after 37 days using 3.62 kg of feed thus giving a FCR of 1.65 (Ross, 2007b). However, the FCR increases with age (Ross,

2007b), in free range systems and production systems located in developing areas of the world FCRs, typically varying between 2 to 4 (FAO 2006).

In the UK, poultry for household consumption was traditionally purchased from butchers' shops, though this has seen a dramatic shift to supermarket purchasing and now less that 6% of poultry meat is purchased from butchers' shops (Magdelaine et al., 2008). Just 11% of poultry meat bought from supermarkets is bought as whole in the UK, with 31% being bought as portioned pieces and 58% being sold as processed products (Magdelaine et al., 2008).

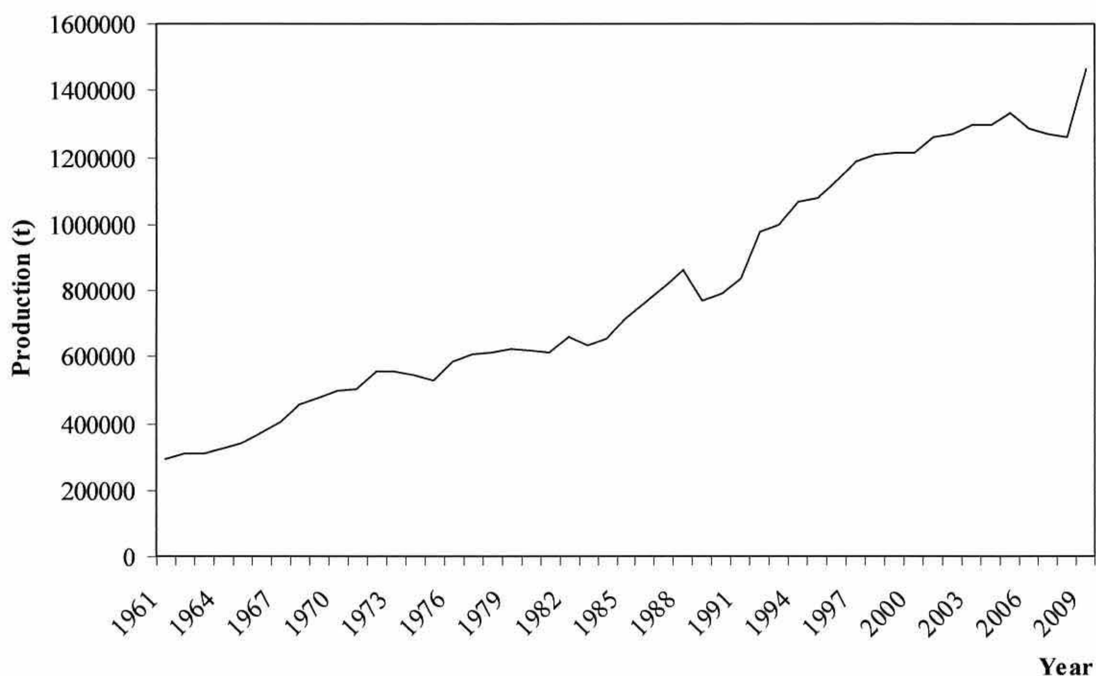


**Figure 1.3** – Trends in UK chicken meat production
Source data: FAOSTAT (2010)

The shift in purchasing patterns within Britain has also coincided with a dramatic increase in poultry consumption, as shown in figure 1.3. To meet this demand the UK broiler industry, like many others, has undergone a mass industrialisation since the 1950s (Kettlewell and Turner, 1985). Production has changed from small-scale supplementary farm enterprises on mixed farms to large scale integrated companies who control not only the rearing process but other aspects of production such as chick supply, feed and processing.

Chicken meat has a very low fat content, typically 4.8% for breast and 11.5% for thigh (Buege et al., 1998), lower than other meats (Juneja et al., 2001). In light of this and other factors such as price and acceptability, increases in production and consumption of chicken meat have occurred. However, it is frequently instigated in cases of food poisoning, leading to interest in the microbiology surrounding poultry production.

### 1.3 Food Poisoning in General

The term food poisoning describes the illness of a human being following consumption of food that contains pathogenic micro-organisms. Although there are a number of these micro-organisms, the types most often associated with food poisoning are *Campylobacter* spp., *Salmonella* spp., *Escherichia coli, Listeria* and *Staphylococcus aureus.* Symptoms arising in the human body usually present themselves as a combination of diarrhoea, nausea, vomiting and stomach cramps (EFSA, 2004; Lee and Newell, 2006). The major and most concerning side affect arising from this is dehydration. Therefore the major treatment for food poisoning is liquids, ideally electrolyte drinks which have greater rates of absorption of water in the digestive system than water alone. Fevers or chills along with headaches and feelings of exhaustion can also be symptoms. Sources of these pathogens include chicken meat and eggs, unpasteurised milk and cheese, shellfish, processed meat (in particular beefburgers and sausages), untreated water sources.

In poultry, these pathogenic organisms live in the bird's gut and/or skin and contaminate the raw meat during slaughter and processing. Under favourable conditions (e.g. warm temperatures), some multiply until cooking. Thereafter, if improperly handled the foods will be consumed and may result in infection. Foods that are consumed raw (e.g. eggs) or are not cooked thoroughly to ensure that the pathogens are eliminated pose particular risk. Indeed, high temperature treatments, such as pasteurisation, have been used for many years to improve the safety of food products (Purnell et al., 2004). Similarly, thorough cooking at temperatures over 70°C eliminates the pathogens (FSIS, 2010); with different time-temperature combinations being effective. For instance, a 7-$\log_{10}$ reduction in *Salmonella* spp. numbers may be achieved after 97 minutes at 55°C, whereas 12 minutes at 60°C and 91 seconds at 65°C achieve the same reduction (FSIS, 2010). Similar results have been shown with many other food-borne pathogens. For instance, Stringer el al. (2000) showed that a 6-$\log_{10}$ 10 reduction in *E. coli* O157 numbers when food was heated to 70°C for a period greater

than two minutes. However, there are a number of factors which can affect the degree of pathogen die-off in meat during cooking. For instance, Juneja et al. (2001) found that the higher the concentration of fat in chicken meat the longer it took to obtain a 7-$\log_{10}$ reduction of *Salmonella* spp. Another factor is meat depth, as outer layers can insulate inner ones. Thus it is the temperature in the middle of chicken meat that is important and techniques such as flash cooking of the skin do not necessarily kill all pathogens.

In addition to inadequate cooking, cross-contamination of foods is another major source of food poisoning; either in storage facilities (products next to one another in fridges) or on preparation areas. An example of this latter instance could be where raw poultry has been diced using a chopping board and knife, and the utensils are then used to prepare a salad without being washed in-between. The salad is consumed raw and therefore infects the person consuming it.

Following ingestion of pathogenic micro-organisms by humans, they enter the stomach. Although the acidity of the stomach kills a proportion, certain pathogens are resistant to this and the chances of survival increase if they are present in large numbers. They next travel through the digestive tract to the intestines where they attach themselves to the walls of the intestines and begin to multiply. There is often a time lag from ingesting the pathogen to the symptoms being shown (the incubation period (CDC, 2006), the length of which is dependent on both the type of micro-organism and the number present. Depending on which pathogen it is, symptoms are caused by either producing a toxin that is absorbed into the bloodstream (CDC, 2006) or through directly invading the deeper body tissues (CDC, 2006). Pathogens such as *E. coli, Salmonella* and *Campylobacter* produce symptoms that overlap with each other. Therefore the only accurate way of knowing the source and cause of a food poisoning outbreak is to conduct laboratory tests.

### 1.4 Prevalence of food poisoning

It was estimated that in England and Wales there are 10.5 million cases of infectious intestinal disease in 1995 of which only 2.4 million could be attributed to directly to the consumption of food (POST, 2003) whereas current estimations for England and Wales in 2007 are 926,000 (DEFRA, 2010). However the recorded number of cases by the Health Protection Agency in 2007 was just 68,962 (HPA, 2010). The discrepancy in data can be attributed to a number of factors. Firstly, cases of diarrhoea and vomiting

are often attributed to food poisoning, though it is thought that approximately half the cases are through contact with the pathogens in the environment (FSA, 2009). Secondly, the difference between the estimated number of cases and the recorded number of cases arises due to only a small percentage of people with the symptoms reporting to a medical facility and having a stool sample taken for laboratory analysis. The FSA recognises that these baseline figures only include a small proportion of the actual cases, as most cases are not confirmed by laboratory testing. However, they believe baseline figures are the most reliable method of monitoring trends in gastro-intestinal disease (FSA, 2006).

The Food Standards Agency (FSA) set a target of 20% (and nearly achieved – 19.2%) reduction in food-borne illnesses in their five year strategic plan 2000-2005 for the five main food-borne bacteria; *Salmonella* spp., *Campylobacter* spp., *E. coli* O157, *Listeria* and *Clostridium perfringens* (FSA, 2004). As well as the benefits to human health benefits, the thinking was that it would lead to significant economic benefits as the costs of food-borne disease to the UK economy is estimated to be £1.5bn per annum (Adak et al., 2002). Following this the FSA targeted a reduction in *Campylobacter* spp. incidence in poultry, with a target of a 50% reduction from there baseline, set at 70%, in UK produced poultry meat 2010 (FSA, 2009) This was not met, indeed the incidence actually increased to 76.1% (FSA, 2009), although the FSA explains this by discussing the difficulty of laboratory isolations via the presence/absence method, and that their new methodology combining this with direct plating is far more reliable.

Interestingly, whilst numbers of *Salmonella* infections have significantly reduced since 1997, Campylobacter spp. infections have only decreased slightly (Figure 1.4). The FSA strategy plan links in with this as the target was to cut *Salmonella* in UK produced chickens between 2000 and 2005. Currently, the target is for a 50% reduction in *Campylobacter* in chickens produced in the UK by 2010 (FSA, 2005). However, although the number of cases of *Campylobacter* spp. in England and Wales declined between 2000 and 2004, they have since started to rise again since 2004 (Figure 1.4).

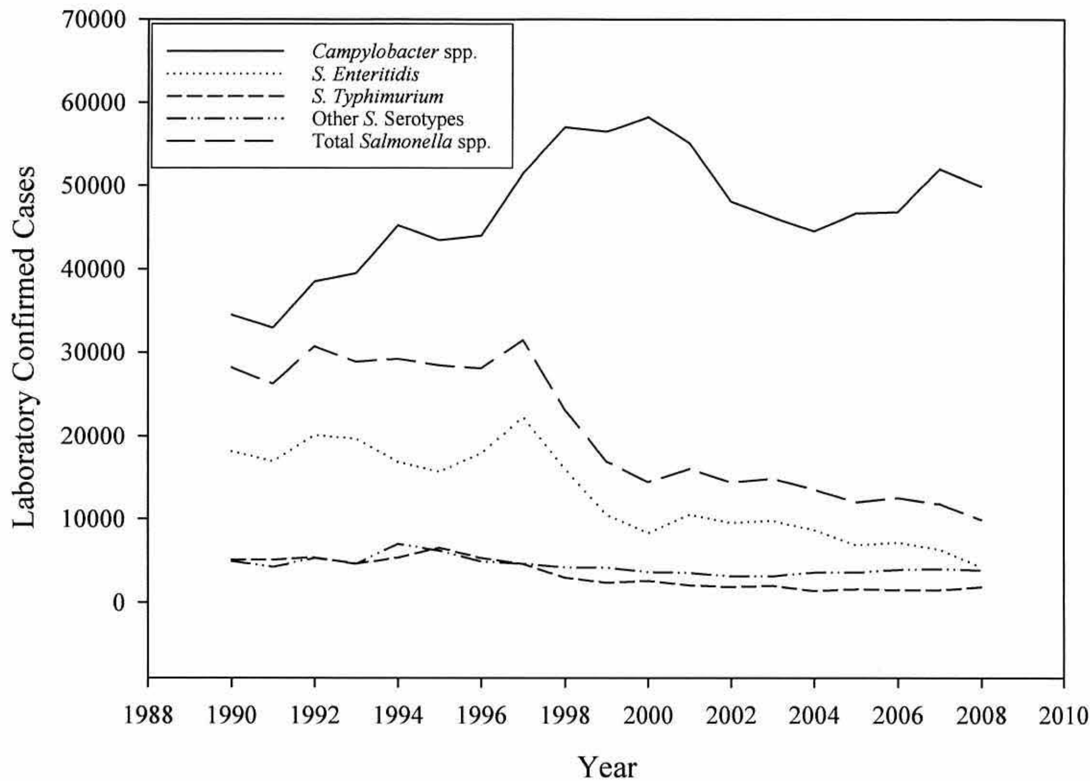


**Figure 1.4** - Trends in *Campylobacter* and *Salmonella* spp. cases in England and Wales, 1990-2008

Source data: HPA, (2009a)

## 1.5 Chicken meat production

Modern broiler production is a highly intensive and integrated process with a number of large integrators responsible for the whole production process (Figure 1.5). Constant monitoring and control of environmental conditions in the broiler house are vital to provide the optimum situation to achieve efficient and rapid growth rate in order that day old chicks can reach the target slaughter weight in the shortest time possible.

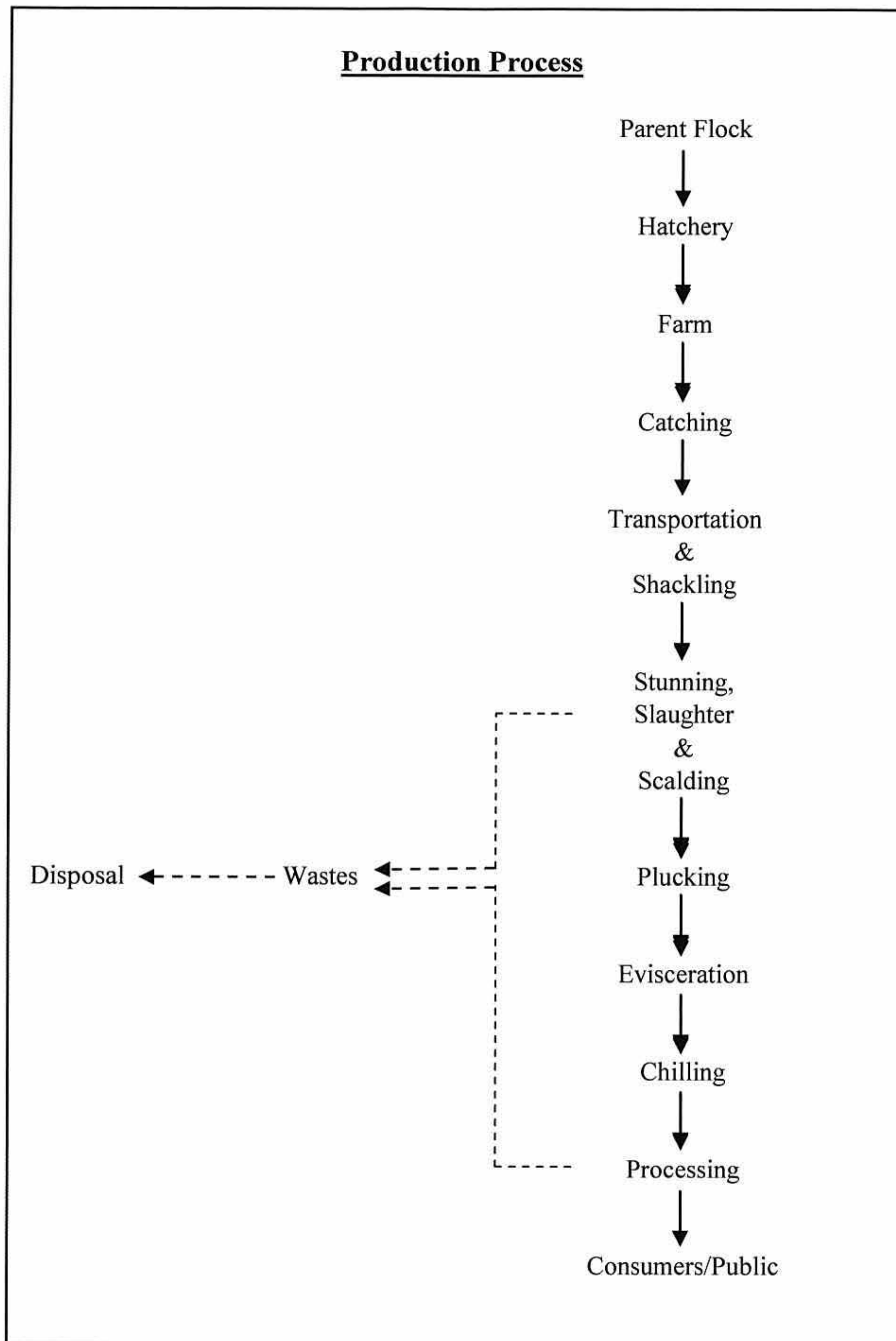


**Figure 1.5** – Broiler Production Flow Diagram

Broiler chicken productions commences with the parent layer flock. Usually kept in deep litter barn conditions on restricted intake rations, hens and cockerels are kept together to produce fertilised eggs. Typically, the hen's and cockerel's feeds are given separately using two separate feeding systems, thus the diets are tailored to suit their respective nutritional requirements.

The fertilised eggs are collected from barn nest boxes, often automatically in order to reduce disturbance for the birds. The collected eggs are stored at 17°C until sufficient

have been collected to form an incubation batch and directly transported to the hatchery. Here the eggs are disinfected with formaldehyde gas in order to sanitise the outside of the shell without damaging the chick embryo inside. After fumigation these eggs are then transferred to incubators which hold the eggs at a constant temperature of 38.5°C and 65% humidity to avoid the embryo drying out for the first 18 days of incubation and for the final three days this temperature is reduced to 37.5°C (M. Wyndham-Lewis, *Personal communication*). The eggs are kept on mechanical racks which turn the eggs roughly every 20 minutes to prevent the embryo sticking to the inside of the shell. After 21 days, the chick hatches (Ross, 2009) and is subsequently collected and vaccinated. Chicks are typically sexed due to the different nutritional requirements for each gender (Ross, 2007b), which in turn leads to the female birds being utilised for smaller, shorter duration (38-40 days) broilers (Ross, 2007b: Ross, 2009), and the males for longer to produce larger birds, typically 3.8 kg at around 52 days of age (Bayliss and Hinton, 1990; Ross, 2007b).

The day old chicks are then transported to the broiler farm. Initially they are often fed from paper placed on the floor bedding (typically sawdust). They are kept at a warm temperature (32°C; Ross, (2009)) which is decreased by around 0.5°C per day until day 20. After this stage the broilers can withstand some fluctuation in house temperature without detrimental effects upon productivity (Ross, 2009) and so the house temperature is permitted to range between 18°C and 20°C depending on climate conditions. This can be used as a tool in hot climatic conditions (to minimise expensive cooling methods) by allowing the temperature to rise slightly during the day, before dropping slightly lower than ideal during the cooler night time.

Water is provided to the broilers *ad libitum* via either bell and/or nipple drinkers. Broilers are also fed *ad libitum* to ensure maximum growth, though the diet of the broilers also changes over their lifespan. For instance, protein levels can be around 23% initially, dropping to 19% by the time of slaughter (Ross, 2009); with amino acid levels following a similar trend (Ross, 2007a). However, the energy supplied by the diet is typically increased from 12.65 MJ/kg at day old to 13.4MJ/kg (Ross, 2007a).

Contaminated feed is a major source of introduction of outside pathogens, particularly *Salmonella* spp. (Heyndrickx et al., 2002; MacKenzie and Bains, 1976). Thus the

careful management of the manufacture, transport and raw materials used are vital factors for successful broiler growth.

## 1.6 Welfare Schemes

As a result of the widespread consumer concerns regarding farm animal welfare (Chilton et al., 2006), and a number of disease events (namely BSE, Foot and Mouth, Avian Influenza outbreaks) a plethora of quality assurance schemes have been created for the chicken industry, focusing on a variety of factors within production. However, whilst some such as the RSPCA's Freedom Foods scheme (RSPCA, 2008) are welfare centred, it has been reported that animal welfare can be compromised as a result of some assurance schemes such as the limited use of medicines in organic production (Hovi et al., 2003) and the stress incurred by livestock being frequently checked for traceability issues (Gregory, 2008).

A number of schemes, such as the RSPCA's Freedom Foods, have set limits for broiler stocking density on the notion that increased densities have a negative effect upon broiler welfare, such as the incidence of footpad and hock dermatitis (Arnould and Faure, 2004). Interestingly though, a number of studies have shown that from a behaviour perspective there was little effect upon individual broiler behaviour (Collins, 2008), and that broilers actually favour the close proximity of other birds (Febrer et al., 2006), often clustering around environmental features within a broiler house (Leone et al., 2007).

## 1.7 Poultry Catching

The catching and transportation of poultry has been said to be the biggest commercial movement of a single type of livestock in the world (Mitchell and Kettlewell, 2004). In the UK, the broiler industry started to grow rapidly in the 1950s (Kettlewell and Turner, 1985) and was one of the first livestock sectors to industrialise (Delezie et al., 2006). However, although automation of broiler production and processing has been widely implemented, there has been little automation of the catching process (Delezie et al., 2006). In most European countries, broilers are still caught by hand and loaded into transportation containers manually (Delezie et al., 2006).

The process begins 8-12 hours (Nichol and Scott, 1990) before loading. Depending on the production system, feed is withdrawn up to a maximum of 10 hours prior to

slaughter to reduce carcass contamination during processing (Bayliss and Hinton, 1990; ACP, 2007; RSPCA, 2008), therefore transportation and holding time must be added into this calculation of time. This temporary starvation means the lower gastro-intestinal tract holds a reduced quantity at the time of slaughter and thus the resultant decrease in pressure means the intestines are less likely to rupture during evisceration (Bayliss and Hinton, 1990). However, other studies (Warriss et al., 2004) have shown that this standard practice increases the moisture content in the intestine and faeces, which in turn leads to more carcass contamination during stunning, slaughter and scalding.

Less than one hour before catching, water is also withdrawn by raising the drinkers to the ceiling (Bayliss and Hinton, 1990; Nichol and Scott, 1990) in order to make the process easier and safer for both birds and catchers. The light intensity is also decreased to near darkness to minimise bird activity (Nichol and Scott, 1990) and reduce stress.

As broilers have been bred for their docility as well as rapid weight gain, they are difficult to drive into transport crates. This is typically done by catching the birds by one or both of their legs, depending on production system welfare guidelines (Kettlewell and Turner, 1985; RSPCA, 2008), and carried to the transportation crates upside down with 3 or 4 other birds in each hand (Delezie et al., 2006). Current Assured Chicken Production (ACP) guidelines (ACP, 2007) prescribe little about how this is to be done, save that the light levels should be low and injured/sick birds are not to be transported. RSPCA freedom foods standards (RSPCA, 2008) stipulate slightly more and state that birds should be caught and carried using both legs (to decrease the forces upon a bird's hip joint) and not more than three birds to be caught or carried in each hand.

Kettlewell and Turner (1985) discuss how gentle handling during catching and loading is needed to avoid bird damage. However, due to the intensive time pressure upon catchers they do not always handle the birds gently, which can cause injuries (De Koning et al., 1987). High levels of bruising can occur with incidence levels as high as 25% being reported (Jaiswal et al., 2005). Jones (1992) concurs, and states how poor handling techniques can cause high incidences of physical injury. This is not confined to visible injury, as the same study found that rough handling (holding upside down by the legs for 30 seconds) increased a bird's susceptibility to tonic immobility and therefore introduced greater levels of fear.

This is an obvious breach of one of the five freedoms of animal welfare and others have discussed how many of the conditions and procedures involved in poultry catching can compromise the bird's welfare (Duncan et al., 1986; Kettlewell and Mitchell, 1994; Scott and Moran, 1993; Stuart, 1980).

Catching is typically carried out during the early hours of the morning when broilers are more docile, in order to supply processing factories for the next working day (Bayliss and Hinton, 1990). These unsociable hours of work are combined with dark (Mitchell and Kettlewell, 2004), hot (20-25°C) and high humidity (≈70%) (Bayliss and Hinton, 1990) working conditions; coupled with high levels of organic dust containing bacterial endotoxins and micro-organisms (Nielsen and Breum, 1995). In addition, the work is repetitive and strenuous, with workers lifting 1000–1500 birds per hour (Kettlewell and Mitchell, 1994; Deliese et al., 2006). Even taking into consideration breaks, lifting 1000 × 2 kg birds every hour for a typical 8-hour shift (Mitchell and Kettlewell, 2004) indicates the severe physical stress and strain upon workers who undertake this occupation.

This all means that the position of hand catcher is considered to be one of the worst jobs within the poultry industry (Jaiswal et al., 2005; Ramasamy et al., 2004). This results in difficulty in recruiting staff (Jaiswal et al., 2005; Mitchell and Kettlewell, 2004) and a tendency to only attract people who are not overly concerned about the methodology employed to catch and load birds (Bayliss and Hinton, 1990). Furthermore, conscientious employees are hard to retain, especially as there is a particularly high incidence of work-related back problems amongst catchers (Mitchell and Kettlewell, 2004).

The problems associated with manual catching and loading of poultry have led to much research being conducted and the development of a number of mechanical harvesters such as the easyload harvesting system (Anglia-Autoflow, Diss, UK) and the Chicken Cat Harvester (JTT ApS, Bredsten, Denmark). These machines use a number of foam-rubber tines on rotating drums to usher birds onto conveyor belts and load the birds into module trays. Mechanical systems are reported to cause birds less damage (Duncan, 2001; Knierim and Gocke, 2003; Lacy and Czarick, 1998) and stress (Duncan et al., 1986) as the birds are not held or inverted (Scott, 1993; Delezie et al., 2007).

Although both manual and mechanical methods inevitably cause short-term stress (Duncan et al., 1986), it has been shown that their heart rate and tonic immobility return to normal more quickly in mechanically caught birds (Delezie et al., 2007). They also have the benefit that they are able to catch at the same pace as a team of manual catchers and yet do not suffer from fatigue and therefore slow down or become less gentle towards the end of a shift (Delezie et al., 2007). However, despite this, public perception has been reported to be the opposite of this (Delezie et al., 2007) particularly in younger, urban females ($P<0.01$).

There are other issues that are limiting the widespread adaption of mechanical catching. These mainly revolve around effectiveness, as for efficient usage they need broiler houses to be clear of support poles (Bayliss and Hinton, 1990; Jaiswal et al., 2005; Ramasamy et al., 2004) in order to be able to move around freely, and higher clearances (of eves and ventilation fans) than those typically found in many older houses (Bayliss and Hinton, 1990; Mitchell and Kettlewell, 2004). This is particularly a problem in the UK where many broiler houses are over 20 years old and of "Queen-post" design, with very few clearspan houses (Mitchell and Kettlewell, 2004). Problems surrounding house design also include the morphology of the house, with long thin houses requiring a great deal of maneuvering and are thus less efficient compared to wide but short houses of the same floor area (Jaiswal et al., 2005). In part this is due to the extra maneuvering room around poles and in part this is because of decreased ferrying distance. This corroborates with other research carried out on an Anglia-Autoflow system, where the distance from the door to the broilers made a considerable difference to the catching times per load (Ramasamy et al., 2004). Again the authors cited post location and maneuverability as the key, and observed that the mechanical catcher rutted wet litter resulting in the catcher occasionally becoming grounded. This necessitated for it to be pulled out, which of course decreased its efficiency.

Other problems have been further reported regarding mechanical catching. Wing damage was found to increase compared to manual catching, though this was balanced by a decrease in leg damage (Ekstrand, 1998); as was un-reliability and expensive maintenance (Lacy and Czarick, 1998; Ramasamy et al., 2004). Furthermore, the designs of such machines often do not lend themselves to ease of disinfection, crucial to prevent transfer of bacteria of diseases such as *Campylobacter* spp., and staff may not

clean the machines as often as is required (Ramasamy et al., 2004). This may significantly increase the risk of introducing diseases and bacteria to the remaining birds (Allen et al., 2008c).

Furthermore the initial high financial outlay for such equipment also works against the adoption of catching machines, as typically broilers are caught on a contract basis thus companies without long-term contracts don't have the security to risk the capital outlay for the machinery. Whilst mechanical harvesters require less physical labour and less personnel (usually 4 per team rather than 7-10 for manual harvesting) the personnel do have to be skilled operators with some degree of training (Jaiswal et al., 2005), and thus rates of pay are higher. It is likely that these problems will be overcome in time, as new broiler houses will be built in designs that promote mechanical catching efficiency, and issues such as reliability and ease of disinfection addressed by manufacturers.

### 1.7.1 Broken bones

The incidence of broken bones in poultry has been investigated by a number of authors. Kettlewell and Turner (1985) found broken bones in 5% of birds sampled, and attributed 20% of this to injuries sustained during catching.

Focusing on wing damage, Andrews and Goodwin (1973) found 13% of broilers sampled at one processing plant had broken wings after plucking. Other studies have compared wing damage between transportation methods and found 11.8% of birds in traditional coups had wing damage (fractures, dislocation and large bruises) whereas more modern open stocked trays, similar to the modular systems now widely used in the UK, had only 6.8% (De Koning et al., 1987). Gregory and Wilkens (1990) found 3% of broilers to have broken wing bones, and classified wing breakage by bone, with approximately 1% of wings having a broken humerous, 2% a broken radius, and 2% a broken ulna; though some wings had multiple breakages. The same study also cites unpublished data from Spackman, which found an average wing bruising of 8.7% and breakage of 2.4% and also stated that whilst there was not enough data for statistical analysis, loads with high levels of mortality seemed to be related to the catching team (also cited by Bayliss and Hinton, 2004).

These variations in breakages could be due to improvements in catching, transportation and processing (stunning and plucking settings in particular); though bird size and age

have also been reported to be factors (Bingham, 1986), with older, heavier birds having higher incidences of wing damage and bruising that younger birds.

Other classes of poultry also suffer from wing damage; for instance the removal of laying hens from cages may give rise to injuries including fractures and dislocation (Kettlewell and Mitchell, 1994). Whilst levels of wing bone breakage are high in broiler production, higher levels of broken bones (29%) have been found in spent hens prior to stunning and 96% post-stunning, illustrating how breakage is exacerbated by levels of osteoporosis in hens after a lengthy period of egg laying (Gregory and Wilkens, 1990).

Additionally, Prescott et al. (2000) measured wing damage in turkeys after unloading from transport crates. They found high levels of fresh blood on the wings (ranging from 3.2% to 17.9% of samples) and lower levels of wing fracture (0.8% to 0%) which they attributed to differences in the catching and handling systems being investigated. Intriguingly, the sample with 17.9% of birds with fresh blood on their wings had 0% fractures and the lowest level of blood had the highest level of fractures, indicating that the two are not necessarily linked.

## 1.8 Transportation

Once the broilers have been loaded into crates they are then transported to the processing plant. Ideally, the plants are located in close proximity to the supplying farms therefore clusters of broiler farms are typically found surrounding a processing plant. In the UK, transportation times rarely exceed three hours (Nichol and Scott, 1990); longer transportation times may occur infrequently, depending upon production requirements and contractual agreements. In addition to the welfare concerns (broilers have no access to food or water during transportation and lairage), large studies have shown a positive correlation between increasing transport times and breast bruising, though strangely a negative correlation between increasing transport times and wing bruising was reported (Carlyle et al., 1997).

As a result of EU directive 71/118/EEC, transportation crates are made out of plastic so they can be easily disinfected after use. However, studies have found factory cleaning of transport crates to be insufficient to remove microbial contamination of *Campylobacter* spp. and *Salmonella* spp. (Allen et al., 2008b). Combined with the high frequency of use, this has been shown to contaminate previously *Campylobacter* spp. negative birds

(Slader et al., 2002). Similarly, Whyte et al. (2001) showed an increase in faecal *Campylobacter* spp. levels and prevalence after transportation. They concluded that the stress induced by the catching and transportation process resulted in extensive carcass contamination. This is further exacerbated by the modular crate design used by the majority of UK producers (Anglia-Autoflow Modular systems, Dis, UK) (Mitchell and Kettlewell, 2004) where the square plastic mesh floor allows faecal matter to drop from upper levels onto birds in lower crates (Stern et al., 1995), and results in the contaminations described in Wilkens et al. (2003).

Bayliss and Hinton (1990) discuss the merits of different types of crates which include loose crates, fixed crates (fixed to a vehicle), and modules. Modules are units kept together and can be in a variety of different forms: multiple floor modules have floors that drop down to create the ceiling of the lower cage and floor of the upper; captive drawer modules are similar but slide out for loading then return once full of birds; unrestrained drawer modules are trays which are filled up with birds, but being open-topped, are very easy to unload at the processing plant (Bayliss and Hinton, 1990). Typically in the UK, modular systems are used, manufactured by companies such as Anglia-Autoflow (Dis, UK) (Mitchell and Kettlewell, 2004). The crate stocking density is determined by total live bird weight (dependent on the breed, sex and age of the birds), weather conditions and the distance to be travelled (Bayliss and Hinton, 1990).

Lairage (the time spent waiting outside the processing plant) is kept to a minimum to help reduce mortality rates. To help with this, trucks are typically parked on the windward side of the plant or ventilated by fans on warm days, and on the lee side on cold days (Bayliss and Hinton, 1990). This has the aim of keeping mortality by suffocation and heat stress to a minimum.

### 1.9 Shackling

Crates are unloaded as soon as possible after their arrival, whilst ensuring the production lines are consistently supplied with birds. Unloading is carried out in a number of similar ways depending on the type of crate used. Common to all is that the birds are removed from the crates and shackled upside down by their feet. The shackles are connected to a chain which moves around the process line.

The process of shackling broilers can also lead to wing damage. Firstly, if the workers who shackle the birds do so in a vigorous manner, this can cause the birds to start to flap their wings, and if severe enough this causes blood vessels in the wings to rupture and gives rise to red wing-tips (Gregory et al., 1989; Jones and Slatterlee, 1997; RSPCA, 2008). In addition, flapping birds can strike their wings against solid objects (e.g. metal stanchions and transport crates) which again would cause red wing-tips (Gregory et al., 1989) as well as bruising and joint dislocation.

Indeed the RSPCA's Freedom Food standards (RSPCA, 2008) recommend that hangers gently run their hands down the legs and body of the bird after shackling as it reduces the incidence of wing flapping. Similarly, a breast comforter has been shown to have the same effect. Jones et al. (1998a and 1998b) showed that hooding birds decreased the incidence of flapping when shackled and although this is not practical in a commercial slaughterhouse, reduction of light levels is possible (Jones et al., 1998a) and/or replacement by blue light which reduces fear in the birds (RSPCA, 2008). From January 2010, RSPCA Freedom Food standards have the additional condition that a bird must not be shackled for more than 30 seconds prior to stunning in an effort to further minimise stress in the broilers.

**1.10 Stunning**

Electrical voltages, administered through a water bath, are typically used to induce unconsciousness in broilers prior to slaughter (Mota-Rojas et al., 2008), although some production plants have investigated and implemented the use of gas stunning.

The aim is for the bird's head and one wing to enter the stun-bath together and give an immediate stun (RSPCA, 2008). However, if a wing-tip touches the water first (e.g. if dislocated during catching or shackling) then the pre-stun shock causes the bird to flap, which in turn encourages other birds on shackles to wing-flap (Kettlewell and Hallworth, 1990). It is imperative that the correct flow of electricity is applied to the birds in the stun-bath as excessively high voltage and current combinations will cause the bird to suffer and induce carcass damage (Gregory and Wilkens, 1990), whilst a combination which is too low will result in the bird being conscious when it is killed or recovery during bleeding (Raj, 1998; 2006).

Damage rates in post-stun broilers found by Gregory and Wilkens (1990) were considerable, with 95.5% of frozen broilers having multiple broken bones (3.4 on average) per carcass. However, in a separate trial, only 3% of birds had broken bones when killed and examined without stunning, though 96% of broilers from the same flock had broken bones after stunning (sampled without plucking or evisceration). Without stunning the percentage of birds with broken bones was; humerus 0%, radius 0.8% and ulna 0.8% though in the post-stunning sample this was 2.8%, 2.0% and 1.3% respectively. This therefore shows that the stunning process is responsible for a significant proportion of wing damage, which is often attributed to other means (e.g. catching) by many in the broiler industry.

**1.11 Slaughter and Processing**

Slaughtering is routinely conducted by revolving knives which slit the neck and the carotid arteries within it, and the bird then bleeds for around 195 seconds (Raj, 2006). By not killing the bird outright at stunning, this allows the heart to keep pumping and helps to remove all the blood from the carcass (Raj, 2006). If not decapitated, once the carcass is bled it goes through an automatic head remover, and from there into a scald tank. The hot water in the scald tank (typically 54°C) softens the feathers which permits easier removal by the de-featherer. This machine has a series of rotating drums on which there are numerous rubber "tines". These mimic the action of a hand and pluck the feathers out of the carcass. Upon exiting this stage, the carcass is inspected by a Plant Inspection Assistant (PIA) to check for any subcutaneous abnormalities. The carcass is then re-hung (automatically) onto a different set of shackles and the feet removed.

The carcass is then eviscerated (the innards removed), which commences with hock removal; the vent is bored and cut, before the machine removes the internal organs and allows them to hang loose for inspection. Any internal organs that show symptoms of disease can be detected during this process and affected carcasses removed from the line. The neck and crop are removed next, and then the carcass is washed inside and outside and again re-hung onto another set of shackles. Finally the wings are inspected and damaged/broken wings are removed, before a final wash and the carcass goes for chilling. The chilling process lasts for 2 hours at temperatures under 4°C.

Once the carcass has been chilled it is weighed and put with others of similar sizes, often by an automated weighing machine. They are then left for 8 hours as the carcass needs to maturate, although this varies depending on customer product specification. Quality control operatives ensure the consignments have been chilled to below 4°C.

Some carcasses are left whole for sale, although many others are processed in to the cuts of meat required by both consumers and processors. The exact nature of the process which separates the cuts of meat varies between different processing plants, due to the level of mechanisation and design of such machines. However the breast, inner fillet, thighs, drumstick, wings, back, wishbone can all be removed and sold as separate pieces of meat. Some, i.e. breast, inner fillet, thigh, are often further processed to produce diced pieces of meat largely for ready-meal manufacture.

### 1.12 Chicken Wings

Wings typically contain 60.7% moisture, 20.9% fat, 15% Crude Protein and 1.6% ash (Crosland et al., 1995). Chicken wings are typically a relatively low-value cut of a broiler carcass, often processed into value added portions (e.g. via breading or using marinade). Whilst their high fat content and gristly nature makes them unpopular in haut-cuisine, the fat content does make them to suitable to certain styles of cuisine, for instance American-style spiced and fried fast food. However, wings have also been shown to pose higher risk of contamination with pathogenic bacteria, such as *Campylobacter* spp. (64.7% prevalence; Flynn et al., 1994), *Salmonella* spp. (40% prevalence; Capita et al., 2003) and *Listeria monocytogens* (Uyttendaele et al., 1997). It is because of this that wings have been used as the subject of a number of microbiological treatment studies such as De Ledesma et al. (1996); Hwang and Beuchat (1995); Kim and Marshall (2000) and Wang et al. (2004).

### 1.13 Microbial Analysis of Poultry Meat

The majority of micro-organisms are found on the outer surfaces of a chicken carcass, particularly the skin, rather than the underlying tissue (Avens et al., 2002; Uyttendaele et al., 1997). These need to be effectively recovered from the samples of meat in order to be analysed.

The typical method for this is to aseptically remove a measured quantity from the sample, typically 10 g for quantitative analysis or 25 g for pathogen detection, e.g.

*Salmonella* spp. For the former, this is placed in to 90 ml of Maximum Recovery Diluent (MRD) to form a 1 in 10 dilution, for either a general or specific bacterial recovery without enrichment (Hutchinson et al., 2006a). Similarly, the 25 g sample is added to 225 ml of enrichment solution, which for *Salmonella* spp. Buffered Peptone Water (BPW) is typically used (Capita et al., 2002; Rasschaert et al., 2007a).

These then can be processed using a stomacher machine (Seward Ltd., Worthing, UK) in order to transfer as many bacterial from the sample into the solution as possible, in a replicable manner (Capita et al., 2002; Hutchinson et al., 2006a; Rasschaert et al., 2007a; Economou et al., 2009), typically for a minute (Capita et al., 2002; Economou et al., 2009) though some samples may require longer (Hutchinson et al., 2006a).

### 1.13.1 Total Viable Counts

Total viable counts (TVC) are used in order to give an indication of the aerobic bacterial load living upon a sample. Standard Plate Count Agar is normally used for this, sometimes spread plated onto set agar (Barakat et al., 2000; Kim and Marshall, 2000) but more typically the sample is pippetted into an empty agar plate before cooled but molten (approximately 46°C) agar is poured in and mixed with the sample (Alonso-Calleja et al., 2004; Hutchinson et al., 2006a; Wang et al., 2004). Whichever technique is used, the plates are incubated aerobically, although the temperature and time may vary depending on individual requirements.

For instance, the International Standard (ISO 4833:2003) has a number of incubation temperatures: 22°C, 30°C and 37°C. Low temperatures such as 3.5°C for 10 days and 22°C for 48 hours have been used for psychrotrophs and mesophiles respectively (Barakat et al., 2000). More typically, warmer temperatures are used such as 30°C for 24 hours (Wang et al., 2004), 48 hours (Economou et al., 2009; Kim and Marshall, 2000) or 72 hours (Alonso-Calleja et al., 2004; Hutchinson et al., 2006a). Others user even higher temperatures such as 35°C for 48 hours (Russell, 2001; Saucier et al., 2000) and 37°C (Whyte et al., 2003) which are clearly more representative of the body temperature of warm-blooded animals.

### 1.13.2 *Pseudomona* spp.

*Pseudomonads* are gram-negative, aerobic, rod shaped bacteria, with uni-polar motility (Ryan and Falkow, 1994). Some species such as *P. aeruginosa* are pathogenic to plants

and humans (Lyczak et al., 2000). Most aerobic, cold-temperature meat-spoilage bacteria are in this genus (Garcia-Lopez et al., 1998), particularly *P. fragi*, *P. fluorescens* and *P. putrefacius* (Ellis and Goodacre, 2001).

Fresh meats are in general slightly acidic (pH 5.5-3.9) and have enough glucose and other simple carbohydrates to support $10^9$ colony forming units (CFU) $cm^{-2}$ (Ellis and Goodacre, 2001). *Pseudomonads* are some of the fastest growers and best utilizers of these when at typical temperatures of refrigeration (around 5°C), hence their predominance on meat products (Ellis and Goodacre, 2001).

Laboratory analysis is typically conducted using serial dilutions of MRD and surface spread onto plates of *Pseudomona* agar with C-F-C supplement (Alonso-Calleja et al., 2004; Economou et al., 2009; Geornaras et al., 1999; Hinton et al., 2004; Hutchinson et al., 2006a; Saucier et al., 2000), incubated at low temperatures (25°C) for one (Alonso-Calleja et al., 2004) or two days (Saucier et al., 2000). The C-F-C supplement contains cetrimide, fucidin and cephalosporin, which are selective agents added to cooled, molten agar as when combined, they permit only *Pseudomona* spp. to grow on the agar, appearing as straw coloured colonies on the surface of the agar with a distinctive blue-green pigment. These can be examined under a Ultra-violet light in order to count fluorescent *pseudomonads* (Alonso-Calleja et al., 2004) and are strongly oxidase positive when tested with oxidase reagent (Jeppesen, 1995).

### 1.13.3 *Staphlococus aureus*

*Staphlococus aureus,* though first identified in 1880 by Alexander Ogston from human wounds, has recently gained a higher media presence by methicillin resistant *Staphlococus aureus* (MRSA) (Washer and Joffe, 2006). However, the bacterium is also an agent of food poisoning (Capita et al., 2001; Cansian et al., 2005).

It is a gram-positive coccus, is facultatively anaerobic, and catalase positive, which can be important to distinguish the bacteria from enterococci and streptococci. *S. aureus* is frequently found on human skin where it usually remains harmless (Washer and Joffe, 2006). It survives for some time in arid environmental conditions and is found in a variety of animals such as domestic pets, horses and poultry. Indeed it has also been reported to be the causal agent of pododermititis in poultry (Wilcox et al., 2009). Not only has it been frequently isolated on poultry carcasses (90.63% in Anower et al.,

2004) but also has been found in broiler house air (50% of samples in Essam-solimon et al., 2009).

Typically *S. aureus* is grown in the laboratory on Baird-parker media with the addition of egg-yolk potassium tellurite emulsion. This combination is widely recommended for this purpose by a host of national and international bodies such as the ISO (Capita et al., 2001; Oxoid, 2010a), and has been used by many authors in scientific research of the bacteria in poultry meat (Alonso-Calleja et al., 2004; Capita et al., 2001; Martin et al., 1989; Saucier et al., 2000; Sirisee et al., 1998). *S. aureus* reduces the tellurite to form grey-black colonies with a surrounding clear halo (Capita et al., 2001; Oxoid, 2010a). Plates are incubated for 24 hours (Alonso-Calleja et al., 2004) and then any negative plates for a further 24 hours (Oxoid, 2010a). Confirmation testing via a latex agglutination kit can then be used to confirm presumptive colonies. A few strains of *S. aureus* are lecithinase negative and thus do not test positive using Baird-parker media with egg-yolk tellurite. However for screening purposes of poultry meat this is not important (Capita et al., 2001).

### 1.13.4 Enterobacteriaceae

Enterobacteriaceae are a family of gram-negative facultatively anaerobic rod-shaped bacteria, typically 1-6µm long and 0.3-µm wide (Singleton and Sainsbury, 1987). Some species are motile (via flagella) and metabolism can be fermentative or respiratory depending on conditions. They can be parasites, pathogens or commensals in humans and animals (Singleton and Sainsbury, 1987). Within Enterobacteriaceae there are a number of well known species such as *Salmonella* (Linton and Path, 1977), *Citrobacter*, *Enterobactor*, *Escherichia*, *Proteus*, *Shigella*, and *Yersina* (Singleton and Sainsbury, 1987). Because of this, Enterobacteriaceae are routinely tested in the food industry to give an indication of possible contamination of pathogens and therefore an assessment of the overall quality of the food and of the hygiene conditions of the processing location (Kornacki and Johnson, 2001). However, whilst these can be an indication of faecal contamination, this is not always the case (Kornacki and Johnson, 2001). Firstly, Enterobacteriaceae are not obligate inhabitants of the gastro-intestinal tract of animals, there are reservoirs within the environment where these organism can exist and indeed they are common in food manufacturing environments, such as the scald tank in poultry slaughterhouses (Geornaras et al., 1998) and it is possible for them to be a constituent of the natural micro-flora of such areas, indeed even in the air (Geornaras et al., 1998).

However, this is particularly pertinent in locations where sanitation standards are not high (Kornacki and Johnson, 2001), and thus do give an indication of poor manufacturing practices and processes.

Enterobacteriacea typically grow as purple colonies on Violet Red Bile Glucose Agar (VRBGA) (Geornaras et al., 1996) as in the intended standard ISO:5552, for 24 hours at 37°C (Economou et al., 2009; Hutchinson et al., 2006b) and are plated out using a pour-plate technique (Capita et al., 2002; Economou et al., 2009; Geornaras et al., 1996; Hutchinson et al., 2006a) as described for TVCs, though once set, a second layer of agar is poured into the plate to create virtually anaerobic conditions whilst allowing fermented gases to escape; however, some (e.g.. Alonso-Calleja et al., 2004) have used this agar as with a spread plate technique. There is also some variation in incubation temperature with 35°C (Capita et al., 2002) and 30°C (Alonso-Calleja et al., 2004; Geornaras et al., 1996) being used in studies.

Finally, statistically significant correlations have been found between Enterobacteriaceae results and other microbiological test results of poultry products such as Total Aerobic Counts and *E. coli* counts in poultry meat (Ghafir et al., 2008) and between Enterobacteriaceae and coliforms in chicken breast skin (Capita et al., 2002).

### 1.13.5 Coliforms

Whilst Enterobacteriaceae are classified as a taxonomic family, coliforms have no such taxonomic validity and are described by Kornacki and Johnson (2001) as a "working concept". Coliforms are lactose fermenting bacteria that often live in the gastro-intestinal tract of warm-blooded animals and are also known as faecal coliforms (Kornacki and Johnson, 2001). Tested using Violet Red Bile Agar (VRBA), which enables only lactose fermenting species to grow. Hence there is overlap with some of the species also being in the *Enterobactera* taxonomic group (Konacki and Johnson, 2001).

Nevertheless, coliform bacteria are still used as indicator organisms for the presence of pathogenic species (Qi et al., 2004), and there is a International standard (ISO:4832) to provide a guide of standard procedures, again using the same pour-plate and overlaying

technique as Enterobacteriaceae (Capita et al., 2002; Hutchinson et al., 2006a; Russell, 2001; Saucier et al., 2000).

Similar to Enterobacteriaceae, coliforms have a variety of possible incubation temperatures, such as 37°C (Javanmard et al., 2006), 35°C (Capita et al., 2002; Russell, 2001; Saucier et al., 2000) and 30°C (Hutchinson et al., 2006a) for times from 24 hours (Capita et al., 2002; Hutchinson et al., 2006a; Saucier et al., 2000) to 48 hours (Russell, 2001; Javanmard et al., 2006). Similarly, some have used spread plate techniques for detection of coliforms (Javarnmard et al., 2006).

Finally, as the bacteria are fermenting bacteria, most probable number (MPN) techniques can be used (Cansian et al., 2005) using Lauril broth sulphate triptosis (LST) or shining area broth, both incubated at 37°C for 24 hours, or EC broth at 44.5°C for 48 hours.

### 1.13.6 *Salmonella* spp.

First isolated and identified in 1885 (Ellermeier and Slauch, 2006), *Salmonella* spp. are bacteria responsible for numerous cases of food poisoning around the world (Carraminana et al., 2004; Smith, 2003a). As discussed earlier, the total number of cases of *Salmonella* infections in the UK has decreased since 1997 (Fig. 2), a pattern that has also been observed in continental Europe (Fisher, 2004). Nevertheless, most European countries still feel it of paramount importance to minimise the number and severity of food-borne *Salmonella* cases (Rose et al., 1999).

*Salmonella* spp. can enter the food chain from a variety of agricultural products; from poultry, beef, pork, through to milk and dairy products, eggs and seafood (Ekperigin and Nagaraja 1998; Sarna *et al.* 2002; Gorman and Adley 2004). *Salmonella* spp. outbreaks in humans are often linked to products containing uncooked eggs such as custards, cream cakes, meringues and eggnog (Madigan et al., 2002) This means the methods used for controlling *Salmonella* need to have a broad scope and not just concentrate on one specific food group.

*Salmonella* spp. are rod-shaped, gram-negative, non-spore forming, facultative aerobic bacteria, (Madigan et al., 2002; Smith 2003a), the majority of which ferment carbohydrates with the production of acid and gas (Smith, 2003a). There are over 2,300

serotypes of *Salmonella* spp. of which >1,400 are known to be pathogenic for humans (Madigan et al., 2002). *Salmonella* spp. typically inhabit the gut of animals and thus are found in their faeces and slurry/sewage (Madigan et al., 2002), and can survive for long periods in the environment (Foster and Spector, 1995). Some strains such as *S. dublin* and *S. choleraesuis* cause disease in animals (cattle and pigs, respectively), but rarely cause corresponding disease in humans (Smith, 2003a); though when they do it is often invasive and life-threatening. Others (e.g. *S. typhi* and *S. paratyphi*) only cause enteric fever in humans and higher primates, though with other *Salmonella* spp. the reverse is true (Smith, 2003a).

Ingestion of between $10^5$ and $10^8$ organisms causes clinical disease in adults (Jay et al., 2005; Madigan et al., 2002), though this is lower for children, elderly, and immunocompromised individuals (Jay et al., 2005), and can be lower in foods with a high fat or protein content, as these offer protection to the organism both within the host and in the external environment (Smith, 2003a). Infective doses result in the colonisation of both intestines between 8–48 hours after the bacteria is introduced (Madigan et al., 2002). Symptoms include the sudden onset of a headache, vomiting, chills and diarrhoea, followed by fever lasting several days (Madigan et al., 2002). After recovery, patients shed *Salmonella* spp. in their faeces for several weeks meaning they remain a source of infection for others, though with some patients this asymptomatic carriage can last for months or even years (Madigan et al., 2002).

*S. enteriditis* and *S. typhimurum* are the two most important strains for salmonellosis in humans, typically causing mild and self-limiting gastroenteritis, though symptoms can be severe in the young, elderly and those with weakened immune systems (Smith, 2003a). Primarily found in poultry, *S. enteriditis* thus predominantly infect humans via eggs and poultry meat (Smith, 2003a). As well as the gastro-intestinal tract colonisation and subsequent contamination of carcasses, the bacterium infects the ovaries of live hens, contaminating the eggs prior to shell formation (Smith, 2003b), and has been isolated from 2.7% of layer flocks and 3% of broiler flocks (Poppe 1994). However, *S. typhimurium* infection can come from a wide-range of human food-stuffs and contact with infected livestock and pets (Smith, 2003a), and the most common cause of *Salmonellosis* in humans (Madigan et al., 2002; Fig. 2).

The widespread use of antibiotics for the control of *Salmonella* spp. has resulted in strains resistant to such treatment (Carraminana et al., 2004), which in turn has led to a number of outbreaks in humans that medical practitioners have struggled to manage (Smith, 2003a). However, although resistant strains are not susceptible to antibiotics, they still respond to treatment with acids, preservatives, drying and disinfectants (Smith, 2003a), meaning good hygiene practice is vital to control the bacterium.

Both contamination of broiler houses prior to chick placement and the vertical transmission to day-old chicks from the broiler breeders have been identified as key routes for broiler infection (Rose et al., 1999). Critical areas of broiler house sanitation have been identified such as walls, feeders, the ventilation system and bedding, with thorough disinfection prior to bird placement combined with efficient rodent control required to reduce the risks of *Salmonella* spp. persistence (Rose et al., 2000). Once within a broiler house, faecal shedding of *Salmonella* combined with coprafelia is the primary method that the infection spread through a flock (Smith, 2003b).

Feed hygiene is also critical, with a variety of heat and/or organic acid treatments being available to sanitise feed, and in the case of organic acids, to protect against colonisation within the bird's gastro-intestinal tract (Van Immeersal et al., 2005). This has been reported to work against the bacteria in two ways. Firstly, undisocciated acid molecules are transported across the bacteria's cell membrane, where the pH change dissociates them, the released hydrogen ions then decreasing the internal cytoplasm pH, which decreases enzyme activity and interrupts cell metabolism. Secondly, it has also been reported that Butyric acid down-regulates the gene responsible for *Salmonella* spp.'s attachment to intestinal epithelial cells, and thus prevents cell invasion (Gantois et al., 2006; Van Immeersal et al., 2004).

### 1.13.7 *Campylobacter* spp.

#### 1.13.7.1 Introduction

It is widely recognised that that *Campylobacter jejuni* is the leading bacterial cause of food-borne gastroenteritis in the US and other developed countries (Debretsion et al., 2007; De Zoete et al., 2007; EFSA, 2004; Evans and Sayers, 2000; Fernando et al., 2007; Skirrow, 1991).

De Zoete et al. (2007) estimate that there are 400 million human cases of entero-colitis worldwide per year as a result of *Campylobacter* spp. infection. The published reported cases are far lower than the actual situation. Skirrow (1991) estimated that whilst at the time the incidence rate was 85/100,000 persons per year, the true rate was probably nearer 1100/100,000 persons per year. Other estimates range between 7.6 and 100 times higher than reported (EFSA, 2004) depending on a country's sampling and reporting regime. The rationale behind these discrepancies is that many cases of *Campylobacter* infection never visit a medical professional, and even of those who do, only a fraction are actually sampled (EFSA, 2004). Additionally, not all positive cases are actually recorded by the system, as not every testing institution is part of a national reporting scheme (EFSA, 2004).

Buzby et al. (1997) estimated the total cost of Campylobacterosis to the US economy to be between $1.5–8 billion per year, and assumed that 55-70% of these infections were food-borne. Thus the cost from food-borne sources of *Campylobacter* would be in the region of $0.8–5.6 billion per year (Buzby et al., 1997). In the last ten years, these figures would have been subjected to inflation, and although slightly mitigated by slightly declining incidence rates. This illustrates why public heath authorities take *Campylobacter* spp. seriously.

Historically, there were major increases in the number of reported cases during the 1980s (Skirrow, 1991), and though in the UK this has somewhat levelled off, the incidence level is still high and fluctuating with current recorded cases in England and Wales of 49,880 in 2008 (HPA, 2009b).

In humans, symptoms of *Campylobacter* spp. infection include watery through to bloody diarhoerea (Lee and Newell, 2006; MacCallum et al., 2005; Mellits et al., 2002; Newell, 2002; Wassenaar, 1997), abdominal pains and fever (Butzler, 1982; Lee and Newell, 2006). Although the disease can be severe in some patients, it is usually self-limiting, occasionally with complications such as reactive arthritis, Guillian-Barre syndrome (EFSA, 2004; Kitchen and Vaughn, 2007; Nauta and Havelaar, 2007) and/or inflammatory bowel disease (Nauta and Havelaar, 2007). Deaths solely due to *Campylobacterosis* are very rare (Smith, 2008). Treatment for the disease in humans usually takes the form of measures to prevent de-hydration, though anti-microbial drugs

such as erythromycin and ciprofloxacin are used for serious cases, although discouraged due to concerns about antimicrobial resistance (Lee and Newell, 2006).

The two groups thought to be at higher risk of infection are those under 5 years of age, and those in the 18-29 years old age range, particularly males (Rosenquist et al., 2003; HPA, 2009a; LDHH, 2007). Conversely, both males and females over the age of 65 years have the lowest risk of contracting the disease. Skirrow (1991) discusses how certain occupations such as farmers, veterinarians, slaughterhouse workers and butchers have an increased risk of infection. However, this is qualified by a discussion about how some level of immunity may be achieved after a number of bouts of *Campylobacterosis* (Skirrow, 1991) in a similar manner to that discussed by other authors (Newell, 2002).

Typically it is *C. jejuni* which is present in commercial poultry, and whilst some authors (Ayling et al., 1996; Berndtson et al., 1996b; Pearson et al., 1993) report low biodiversity within the same flock, others report that there are often multiple genotypes even in the same bird's gastro-intestinal tract (Hook et al., 2005; Stern et al., 1997) due to multiple environmental exposures, which have been reported to persist in subsequent samples including ones taken at slaughter (Hook et al., 2005). Interestingly, EFSA (2004) report that certain rearing conditions, such as free range, give rise to greater biodiversity and higher flock prevalence than more "*industrialised*" chicken production.

*C. jejuni* is also the predominant strain of *Campylobacter* spp. isolated from human *Campylobacterosis* cases, reported to be up to 90% of cases (EFSA, 2004). The majority of the remaining cases are accounted for by *C. coli*, which is predominantly found in pigs (EFSA, 2004; Skirrow, 1991).

A number of risk factors have been identified for human infection. Predominantly this is a result of cross-contamination from mishandling raw meat (EFSA, 2004; Ghafir et al., 2007; Rosenquist et al., 2003; Vellinga and Van Loock, 2002), especially turkey, duck and chicken, but to a lesser extent, pork, beef (EFSA, 2004; Ghafir et al., 2007; Whyte et al., 2004), lamb (Whyte et al., 2004) and veal (Ghafir et al., 2007). Whilst ruminant and pig meat are rarely contaminated (Ghafir et al., 2007; Whyte et al., 2004) undercooked offal, especially livers, from these animals is thought to be a considerable risk (EFSA, 2004 (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2006),

Additionally, commercially prepared food (Vellinga and Van Loock, 2002), barbequed food, especially sausages (Vellinga and Van Loock, 2002), water from untreated sauces (EFSA, 2004) and other foods such as raw milk (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2004), oysters, mushrooms and pâté (all Whyte et al., 2004) have been identified as sources of *Campylobacter* spp. infection. Finally, handling animals, both farm and domestic pets, has been suggested as a risk factor for humans (Vellinga and Van Loock, 2002) as both types of live animals can potentially be carriers of *Campylobacter* spp., often displaying no visible symptoms.

The climate/season has been identified as a factor influencing the prevalence of human infection in humans, as prevalence is highly seasonal with the peak incidence occurring in late spring/early summer (EFSA, 2004, HPA, 2009b). Indeed, the distribution of human *Campylobacter* spp. infections has been proposed to be related to the growth cycle of one or more insect species and their access to environmental sources of *Campylobacter* spp. (Nichols, 2005).

The infective dose for humans is variable, depending on the previous exposure, and the immune status of the individual. Some authors report that the infective dose for humans can be as low as 500 CFU (Robinson, 1981; Newell, 2002); although Newell (2002) also mentions how in some individuals doses as high as $10^9$ CFU do not always cause illness. Interestingly, repeated exposure in humans seems to lead to protection (at least in part) from the effects of the disease, though not from colonisation of the individual's digestive tract by the bacteria (Newell, 2002). These people therefore are asymptomatic carriers in the same manner as many animals.

#### 1.13.7.2 Description of the bacteria

*Campylobacter* spp. are gram stain negative, oxidase positive, spiral rod-shaped bacteria, 0.5-0.8 μm long and 0.2-0.5 μm wide (Berndtson et al., 1992; EFSA, 2004). Most species have a flagellum on at least one end of the rod, which it uses to create it characteristic "corkscrew" movement (EFSA, 2004). This motility is required for the bacteria to reach the attachment sites in the gastro-intestinal tract, and for the penetration into the intestinal cells (Wassenaar, 1997); though Newell (2002) disputes this and states there is little or not evidence of attachment to the under-lying cells in the host. *Campylobacter* spp. obtain their energy from amino acids or tri-caboxylic acids rather than carbohydrates (EFSA, 2004).

All *Campylobacter* spp. thrive in the conditions found in the gastro-intestinal tract of animals (43°C in birds, 37°C in humans; Newell, 2002) and indeed it only reproduces in these conditions (Newell, 2002). It is neither an aerobic or anaerobic bacteria, as it is susceptible to normal levels of oxygen found in the air and grows best at between 3% and 15% oxygen and 3% to 5% carbon dioxide (Krieg and Hoffman, 1986). It is therefore termed a microaerophilic bacteria (Kaaloush et al., 2007) that requires low levels of oxygen, as found in animals' gastro-intestinal tracts. Kaaloush et al. (2007) show that *C. jejuni* is an obligate microaerophile and found that different strains had different oxygen tolerances.

This is relevant to the practical control of the bacteria in poultry as broiler farms have many environmental niches (e.g. cracks in house walls), which when combined with poor hygiene practices prior to chick placement, could be a route of infection from one flock to subsequent production cycles (Evans and Sayers, 2000).

*Campylobacter* spp. are susceptible to arid conditions, and somewhat to low temperatures, though not all species are killed by freezing (EFSA, 2004). Indeed, *Campylobacter* can remain viable for long periods in water and foodstuffs (Newell, 2002). Similarly, Skirrow (1991) reports how *Campylobacter* many survive for several weeks in cold water, but only a few days in warmer water (>15°C).

Once in the gastro-intestinal tract of the host animal, the bacterium grows in the mucus overlying the intestinal epithelial cells – maintaining their position in this flow by using their flagella for motility (Newell 2002). *Campylobacter* spp. produce a variety of toxins; Enterotoxins and Cytotoxins (McFarland and Neill, 1992; Newell, 2002; Wassenaar, 1997), although not all strains produce both (Newell, 2002; Wassenaar 1997) and the toxigenic profile of these is complex (McFarland and Neill, 1992). Enterotoxins are "*secreted proteins with a capacity to bind to a cellular receptor, enter the cell and elevate intracellular cyclic AMP levels*" (Wassenaar, 1997). Cytotoxins (also known as Cytolethal Distending Toxin, CDT) however are proteins that kill the intestinal cells (McFarland and Neill, 1992) by acting intra-cellularly, causing distension and eventual cell death (Newell, 2002) or forming pores in the cell walls (Wassenaar, 1997) allowing the contents to leak out. CDT production varies between strains, and CDT negative strains of *Campylobacter* spp. have been isolated from diarric

faeces, which would suggest that CDT is not essential for the symptoms of enteritis (Newell, 2002). Others (Wassenaar, 1997) discuss how enterotoxin production results in water diarrhoea, whereas cytotoxin production causes bloody diarrhoea (coming from the intestinal lining cell damage), though whilst this correlation was found in some studies, it was not in all. It would seem that the purpose of toxin production is to damage cell integrity in the host, and thus enable the leakage of the cell contents in order to supply the bacteria with essential nutrients such as iron, (Newell, 2002).

In some cases the bacteria migrate from the intestine and colonise other organs within the host's body such as the liver, hence why animal livers and other offal are thought to have a higher risk than other parts (EFSA, 2004; Whyte et al., 2006). It has been suggested that this extra-intestinal invasion of the host may play a role in the self-limitation of the disease as it induces a quick and sizable response in the host animal's immune system (Newell, 2002).

**1.13.7.3 Causes in Humans**

Animals have been reported to be the principle reservoir from where human infection originates (Skirrow, 1991). Transmission can be either direct or indirect. Examples include contact with live animals, the environment, transmission by vectors such as insects (Nichols, 2005), consumption of undercooked meat products and cross-contamination from meat products due to poor hygiene practices (Evans and Sayers, 2000), together with consumption of other foodstuffs including raw milk, (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2004), shellfish, and offal, especially livers (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2006).

Of these, poultry meat has been highlighted as one of the principle causes of human cases of *Campylobacter* spp. infection, from contaminated ready-to-eat foods, direct hand-to-mouth transfer and cross-contamination onto raw food during preparation (EFSA, 2004). A lesser risk comes from undercooked poultry meat (EFSA, 2004), particularly poultry offal such as liver, where both bacterial numbers and prevalence is high (Whyte et al., 2006), though is similar to other animals' offal, particularly liver (EFSA, 2004), which would suggest the associated risks are comparable.

In 1999, the Belgian dioxin crisis gave an opportunity to assess the impact of poultry meat on human cases of *Campylobacter*. For four weeks fresh poultry meat was withdrawn from sale throughout Belgium, and during that time the Belgian

*Campylobacter* surveillance system reported approximately a 40% decrease in human cases (Ghafir et al., 2007; Vellinga and Van Loock, 2002). When fresh poultry returned to sale, the infections retained to levels comparable with the previous years (Vellinga and Van Loock, 2002). Interestingly, they also report that infections of *C. coli* actually increased slightly during the crisis, and as pork remained on sale it is probable that this is a reflection of chicken consumption being replaced by pork consumption during this time. Additionally, it is worth mentioning that imported frozen poultry was still available for purchase during the crisis, which would indicate that freezing is an effective method of control. Similar experiments have shown freezing gives a reduction in *Campylobacter* spp. numbers on a carcass by approximately 1.5 $\log_{10}$ units (Rosenquist et al., 2006). This method is actually used as a method of control in Denmark, Iceland and Norway, where flocks which test positive for *Campylobacter* spp. are allocated for the production of frozen chicken meat (Rosenquist et al., 2006).

#### 1.13.7.4. *Campylobacter* spp. in Poultry Flocks and Products

Poultry have been identified as one of the principle sources for human cases of *Campylobacterosis* (Evans and Sayers, 2000; EFSA, 2004). Indeed, poultry typically have high levels of *Campylobacter* spp. both on and in them, and whilst the bacterium grows favorably within their gastro-intestinal tract, they are in fact asymptomatic carriers, suffering little adverse effects to the bacteria being present (Newell, 2002).

In countries such as the Netherlands, it has been estimated that poultry account for 20–40% of all cases of the *Campylobacterosis* in humans (Nauta and Havelaar, 2007). Surveys of UK retail poultry meat have isolated the bacteria from >65% of sampled UK poultry meat (FSA, 2009; Harrison et al., 2001; Jørgensen et al., 2002; Meldrum et. al., 2005a).

**Table 1.3** – Reported prevalence of *Campylobacter* spp. in retail meat (%)

| Retail Sampling | n | Chicken Meat Sample | | | Country |
|---|---|---|---|---|---|
| | | General | Whole Birds | Breast Fillets | |
| Cui et al., (2005) | 61 | 74 | | | USA |
| Dominguez et al., (2002) | 198 | 49.5 | | | Spain |
| Ghafir et al., (2007) | 687 | | 21 | 12 | Belgium |
| Harrison et al., (2001) | 300 | | 68 | | Wales |
| Jørgensen et al., (2002) | 241 | | 83 | | England |
| Kinde et al., (1983) | 94 | 82.9 | | | USA |
| Meldrum et al., (2005a) | 736 | | 73.1 | | Wales |
| Sallam (2007) | 170 | 64.7 | | | Japan |
| Whyte et al., (2004) | 890 | 49.9 | | | Ireland |

High levels of prevalence of *Campylobacter* spp. have also been isolated from retail meat throughout the western world (Table 1.3), and similarly, high levels of broiler flock infection (Jacobs-Reitsma et al., 1994, 1995, Evans and Sayers, 2000, Hald et al., 2000, Heuer et al., 2001, Barrios et al., 2006). This high level of prevalence in the raw meat, combined with their susceptibility to being mishandled in the course of food preparation by the consumer (Uyttendaele et al., 2006) has lead to a focus on reducing the prevalence of *Campylobacter* spp. in live birds, and targets for this reduction being set; a stated "*50% reduction in incidence of UK produced chickens testing positive for Campylobacter by 2010*" (FSA, 2008).

Whilst some authors (Uyttendaele et al., 2006) have commented that a "maximum acceptable level" being set by the relevant food safety authorities would stimulate the broiler industry into action against the bacteria, the EFSA (2004) have stated that the setting of retail poultry microbiological standards for *Campylobacter* would not be cost-effective as it would impose unnecessary testing of retail products. However EFSA (2004) also state that the reduction of numbers of *Campylobacter* spp. in birds and on

broiler carcasses would considerably decrease the risk to consumers. If this is the case, then the question is raised about how any reduction is to be monitored. Rasschaert et al., (2007b) discuss how flock prevalence within Europe varies greatly, ranging from 3% in Finland to more than 90% in the UK. A survey of literature for the prevalence of flock colonisation (summarised in Table 1.4) seems to support this.

**Table 1.4** – Broiler flock prevalence of *Campylobacter* spp. in a number of European countries (%)

| Study | Number of Flocks Sampled | % Positive for *Campylobacter* spp. | Sampling Method | Country |
|---|---|---|---|---|
| Barrios et al., (2006) | 1091 | 15 | Caecal | Iceland |
| Evans and Sayers, (2000) | 100 | 81.6 | Cloacal Swabs | UK |
| Jacobs-Reitsma et al., (1994) | 187 | 82 | Caecal | Netherlands |
| Rasschaert et al., (2007b) | 56 | 73 | Gastro-intestinal tracts | Belgium |
| Refregier-Petton et al., (2001) | 75 | 42.7 | Fresh Droppings | France |
| Russa et al., (2005) | 1737 | 40.9 | Caecal | Netherlands |
| Tuyttens et al., (2008) | 14 | 55.7 | Caecal | Belgium |
| Wedderkopp et al., (2000) | 4286 | 46 | Cloacal Swabs | Denmark |

The figures in Table 1.4 omit some interesting points. For instance, whilst Jacobs-Reitsma et al., (1994) found an overall prevalence of 82%, the rate was 100% in the June to September period, but only 50% in March.

However, flock prevalence rates throughout Europe vary dramatically, from 3% in Finland to more than 90% in the UK (Rasschaert et al., 2007b); which could in part be due to differences in climatic conditions. Peak prevalence occurs at the end of summer and beginning of autumn in moist, temperate countries (UK, Belgium, Netherlands and Northern France; (Bouwknegt et al., 2004; Jacobs-Reitsma et al., 1994; Refregier-Petton et al., 2001) as opposed to the hot and dry months earlier in the summer, in part probably due to the bacteria's susceptibility to arid conditions (Bell, 2006; Corry and Atabay, 2001).

Similarly, Barrios et al., (2006) tested 1091 Icelandic broiler flocks at slaughter between May, 2001 to December, 2003. Overall, it was found that 15% of the flocks had colonisation by *Campylobacter* spp., though most (95%) of the infected flocks were raised during April–September of each year. These would both seem to support the reported seasonality effect discussed previously in this literature review.

Other authors (Table 1.4) have reported other details that are masked by the crude prevalence at slaughter. Evans and Sayers (2000) found differences over time for the same flocks, with increasing prevalence with age. Of 100 broiler farms tested, they found 40% were positive at 4 weeks of age, but 90% at 7 weeks of age (typically just prior to slaughter) – though some flocks were not grown to this age, thus biasing the figures.

Russa et al. (2005) found the overall prevalence to be 55.7%. Again, this masks a number of effects, namely the effect of thinning operations and different production company. For example within one producer's results, the prevalence was 55.7 % in thinned flocks and yet 34.0% in flocks without thinning. This said however, when these were combined with a second producer's results and other factors taken into consideration (namely age and season), it was reported that there was an absence of a significant association between thinning and the risk of *Campylobacter* spp. colonization when tested at the end of the production cycle.

#### 1.13.7.5 Sources of *Campylobacter* spp. infection in broilers and associated risk factors

There are many potential sources and methods of infection for broilers, ranging from workers (Evans and Sayers, 2000; Kapperud et al., 1993) insects (Berndtson et al., 1996a), rodents (Arsenault et al., 2007) and drinking water (Pearson et al., 1987, 1993). Indeed, Bolder (2007) states that whilst in a working poultry farm, the production of *Salmonella*-free birds is feasible, even in "*high-containment*" facilities such as broiler breeder farms, it is near impossible to stop *Campylobacter* spp. from entering and infecting the birds. This is in part due to the low infectious dose (10 CFU) needed to infect one bird, (Newell, 2002) – though typically around 100 CFU would be required (Fernando et al., 2007). This contrasts with a reported varience in poultry for *Salmonella enteritidis* of 6500 CFU to 5.6x10$^4$ CFU (Holt, 1993). Once infected, rapid colonisation (within a week; Hald et al., 2001) occurs within the whole flock (Evans

and Sayers, 2000); e.g. through contaminated litter (Kazwala et al., 1992; Montrose et al., 1985). Thus it has been reported that within flocks birds have either a near 100% infection rate or are not infected at all (Rushton et al., 2009). Similarly, other studies have reported that broiler flocks become colonized with *Campylobacter* spp. around 3-4 weeks of age with isolation percentages of 100% (Jacobs-Reitsma et al., 1995).

### 1.13.7.5.1 Age of Slaughter

Similarly, Arsenault et al., (2007), Berndtson et al., (1996b), Bouwknegt et al., (2004) and Russa et al., (2005) have all reported that as the age of the birds at slaughter increased so too did the risk of the birds being colonized by *Campylobacter* spp.. Arsenault et al., (2007) suggest that this could be caused either by an increase in exposure time or an increase in probability of detecting infection. Strangely however, a large survey of 57,000 broilers over 4286 flocks found no association between slaughter age and *Campylobacter* spp. colonisation (Wedderkopp et al., 2000).

### 1.13.7.5.2 Broiler Breed

Bouwknegt et al., (2004) found that the breed of broiler also affected the prevalence of *Campylobacter* spp. They found that with Ross breeds the prevalence was 25%, Cobb was 23% and Ross-Cobb hybrids had a slightly increased level of prevalence (28%). However other breeds had a markedly increased prevalence of 39%. Plausibly these differences could be due to a correlation between production system and breed as Ross and Cobb are typically used in intensive systems, and not in more extensive systems such as free-range, or it could be that the sampling methods employed biased the focus onto Ross and Cobb breeds as over 92% of birds tested were from these two breed types.

### 1.13.7.5.3 Production System

The type of production system employed has been reported as affecting the prevalence of *Campylobacter* spp. Arsenault et al. (2007) found that greater numbers of birds raised on a farm increased the chance of colonization of ceacal content. They also found a high correlation between the chances of colonisation and the number of broiler houses at a location, which agrees with the work by Refregier-Petton et al. (2001) and Bouwknegt et al. (2004). Arsenault (2007) suggests that this is because birds from other flocks raised in the same production site are likely to act as reservoirs of the bacterium, followed by inter-flock transmission by workers, rodents or insects.

Organic production has been found to have higher levels of prevalence than conventional production systems (Cui et al., 2005; Heuer et al., 2001; Luangtongkum et al., 2006); with one study isolating the bacteria at 100% for organic, 37% conventional and 49% for extensive indoor broiler flocks (Heuer et al., 2001). However, the slower growth rates observed in organic broiler production could have some effect on this, as increasing age increases the risk of infection, and organic broilers are typically older at slaughter compared to conventional birds (Heuer et al., 2001; Luangtongkum et al., 2006). Interestingly, this latter study found that although the prevalence of *Campylobacter* was greater in organic chicken, the isolate resistance to fluroquinolones was less than 2% in the organic samples whereas over 46% of conventional *Campylobacter* isolates were resistant. Other antimicrobial drugs had similar results. They explain this difference in resistance as being due to the routine use of antimicrobials in conventional production and absence of usage in organic systems. Conversely, Tuyttens et al. (2008) found no difference in the prevalence of *Campylobacter* spp. between organic and conventional production systems, though they investigated only a limited number of flocks in their study. Additionally, in the author's opinion there are further limitations on the validity of their findings as the breeds differ between systems and so too does the flock size (average 18,878 for conventional, 4179 for organic), both of which are reported by various other authors as being risk factors in their own right.

The possibility of *Campylobacter* spp. carry-over from previous flocks has been suggested by a number of authors including Evans and Sayers (2000), particularly if house cleansing is incorrectly carried out. However, environmental sampling after cleaning but prior to chick placement has failed to isolate the bacteria (Berndtson et al., 1996a; Evans and Sayers, 2000), and similarly from fresh, clean litter prior to chick placement (Jacobs-Reitsma et al., 1995) Similarly, Refregier-Petton et al. (2001) couldn't identify any significant relationship between prevalence rate and hygienic practices or biosecurity measures. These factors included the use of detergent, number of house disinfections and empty period of house which all relate to the carry-over from previous flocks. This could in part be due to the disinfections being adequate on the research farms, or could be (as suggested by the authors) that biosecurity measures were not strictly enforced and thus contamination from the environment was rife.

#### 1.13.7.5.4 Other Animals

A number of authors have reported that keeping other animals, both domesticated and farmed, within the vicinity of broiler sheds increases the risk of colonisation and prevalence. Kapperud et al. (1993) and Van de Giessen et al. (1996) found that an association with tending other farmed animals such as pigs and other free-range poultry were both independently associated variables, as was the presence of other farm animals such as cattle and sheep.

Although rodents have been suggested as vectors of *Campylobacter* spp. by a number of authors (e.g., Kapperud et al., 1993; Berndtson et al., 1996b; Gregory et al., 1997), others (Evans and Sayers, 2000) found rodents not to be a source of infection though most sites studied operated effective vermin-control programmes. Interestingly, both Evans and Sayers (2000) and Arsenault et al. (2007) found an increased risk (decreased median number of days to infection) in situations where rodent control was operated by an external contractor rather than site staff. Arsenault et al. (2007) suggested that this could be for one or more reasons: that only professional controllers were called in when infestations were severe, that the exterminators themselves spread the bacterium or that the observed results could be a residual confounding effect caused by size of farm as larger farms have a greater reliance on outside contractors and larger farms are in themselves an increased risk factor.

Insects have been shown to be a potential vector for the transmission of *Campylobacter* spp.. Shane et al. (1985) demonstrated that *C. jejuni* could be isolated from houseflies *(Musca dornestica)* after being confined for 5 days in a Horsfall isolator containing 25-day-old, *C. jejuni* contaminated chickens. The study then showed that when contaminated flies were transferred into a second isolater containing Specified Pathogen Free (SPF) chickens, these became infected with *C. jejuni*. Furthermore, they report that the bacteria could be recovered at rates of 20% from the insects' outside surfaces, but 70% of the viscera. More recent studies have shown that the prevalence of *Campylobacter* spp. in flies varies with both time and temperature (Skovgard and Hald, 2007). The prevalence declined both with time ($P < 0.01$), increasing temperature ($P < 0.01$) and decreasing bacterial dose ($P < 0.01$), and concluded that the duration of *Campylobacter* spp. carriage was less than 24 hours at temperatures above 19.5°C. This therefore would be more than enough time for an insect to become infected within one

broiler house and transfer the bacteria into another house either on the same broiler unit or even nearby farm.

#### 1.13.7.5.5 Climate

It has been reported by a number of authors that climate is another independent variable which affects colonization rates of *Campylobacter* spp. in poultry. Climate varies by both location (geographic region) and season, and both of these have been reported to effect colonization rates (Kapperud et al., 1993). Many studies have found a summer and early autumn peak in colonisation rates (Bouwknegt et al., 2004; Heuer et al., (2001); Jacobs-Reitsma et al., (1994); Refregier-Petton et al., (2001); Russa et al., (2005); Skirrow (1991); Wedderkopp et al., 2000). However, conversely Evans and Sayers (2000) did not find any relationship between time to infection and season, although they did comment that they found the lack of seasonality surprising. Although it can be argued that many broiler rearing sheds are climate-controlled units and therefore the environment within relatively constant throughout the year and throughout locations, this has an impact upon *Campylobacter* spp. in the environment in terms of their survivability and transmission by vectors such as insects (Hald et al., 2007; Nichols, 2005).

#### 1.13.7.5.6 Environment

A number of authors determine that the environment is a major source of *Campylobacter* spp. (Arsenault et al., 2007 and Jacobs-Reitsma et al., 1995). At the macro-scale this includes lakes and rivers (Schaffter and Parriaux, 2002; Skirrow, 1991), as although the bacteria can only survive for a few days in water above 15°C, it can persist for a number of weeks in colder waters (Skirrow, 1991).

However, the immediate area surrounding broiler houses (e.g. rainwater puddles, driveways and the houses themselves (Allen et al., 2008c) are far more likely to provide sources for infection via vectors such as workers, visitors, catchers and equipment. For instance, children entering a broiler house have also been identified as a source of infection (Bouwknegt et al., 2004) though plausibly this is due to them not following biosecurity procedures as thoroughly as an adult (e.g. boot dips), rather than being actual sources of *Campylobacter* themselves.

The type of production system employed in rearing broilers can have an effect upon the risk of pathogen introduction as certain systems such as free-range, have a greater opportunity for infection from external sources (e.g. wild birds, contaminated ground).

The air within the broiler house could also lead to cycling of the bacterium in the environment and between birds. Berndtson et al. (1996a) found 43% of air samples positive for *Campylobacter* in infected houses, and yet 0% from uninfected houses. Whilst air could be a symptom of infection rather than a source, others have discussed the problems surrounding air within a broiler house, particularly in warm summer months, and mention how the increased temperatures and low oxygen conditions create an environment favourable to *Campylobacter* spp. (Refregier Petton et al., 2001).

Simmons et al. (2001) found that whilst collected rainwater contained a variety of pathogens such as *Salmonella* spp., the same study failed to isolate any *Campylobacter* spp. Similarly, another study was not able to isolate the bacteria from any of the drinking water samples taken from broiler houses which were positive for *Campylobacter* spp. (Berndtson et al., 1996a). Potentially this is due to difficulties in laboratory techniques rather than the lack of the bacteria; however, as a number of studies have shown *Campylobacter* spp. to be viable but not cultureable (Chaveerach et al., 2003; Jones et al., 1991; Rollins and Colwell, 1986). Other studies using direct PCR detection found similar limitations with culture methods, reporting prevalence of 68% from 231 samples of sand from chicken accommodation, though just a 4% detection using traditional culture methods on the same samples (Studer et al., 1999). Similarly, environmental sampling of broiler houses prior to chick placement has failed to isolate the bacteria from both cleaned and uncleaned broiler houses (Berndtson et al., 1996a), and from fresh, clean litter prior to chick placement (Jacobs-Reitsma et al., 1995) using conventional culturing techniques.

#### 1.13.7.5.7 Thinning

Whilst conventional standards of production have a flock confined to indoors, which are therefore easier to manage and maintain a strict biosecurity regime, they also typically utilise the process of thinning – the removal of a proportion of the birds for early slaughter and growing those left onto heavier slaughter weights. The entering of the shed by a high-risk group of the population, namely catchers (Allen et al., 2008c) gives the potential for them to transmit infections to the birds left in the shed after thinning.

Studies have routinely isolated *Campylobacter* spp. on the bodies of workers, the inside of their vehicles, their equipment (e.g. forklift trucks) and transportation wagons (Allen et al., 2008c). This would tally with other findings such as Hald et al. (2000, 2001) and Jacobs-Reitsma et al. (2001) which highlighted the thinning process as a major risk factor for *Campylobacter* spp. colonisation.

However, this is at odds with Russa et al. (2005) who found no association between the practice of thinning flocks and colonisation by *Campylobacter* spp. in 1737 Dutch broiler flocks. This paper criticises both other authors' studies due to their sampling bias and states that the age of the flock is the over-riding variable. Whilst this may well be true, it ignores the fact that catchers are one very plausible pathway of infection and as they work on numerous farms, often within the same day, they could easily transmit the bacteria if correct hygiene practices are not followed, especially as equipment is difficult to clean and disinfect (Allen et al., 2008c).

**1.13.7.5.8 Final Harvesting including Feed Withdrawal**

The effect of feed withdrawal on the increased prevalence of *Campylobacter* spp. on carcasses has been documented (Northcutt et al., 2003). This is due to contamination from crop contents, which are far more likely to rupture than caeca (Hargis et al., 1995), increasing the possibility of carcass contamination. Feed withdrawal significantly increased the prevalence of positive *Campylobacter* isolates (Byrd et al., 1998) and yet the study found no significant change in ceacal content prevalence. The reasons behind this include the change in crop environment after feed withdrawal, which becomes less acidic and more favourable to *Campylobacter* spp. (Bolder, 2007), although Byrd et al. (1998) points out that this could also be from ingested contaminated litter which birds use as a feed substitute during the withdrawal period. The consistency of the gastro-intestinal tract contents change too, becoming less viscous which increases the chances of leakage (Bolder, 2007). Thus the higher levels of *Campylobacter* spp. post feed withdrawal combined with fact that the gastro-intestinal tract contents change in consistency means that there are greater chances for contamination during processing, and yet feed withdrawal remains a standard practice in the broiler industry.

Catching has similar biosecurity to the process of thinning, with catchers, transport crates and vehicles all possible vectors for spreading pathogens. However, the relative

importance is perhaps changed slightly, as at flock clearance the issue of contaminating other birds is not relevant; whilst crate hygiene however is important to prevent the introduction of pathogenic bacteria into previously negative birds en route to the processing plant (Allen et al., 2008a and 2008b; Berrang et al., 2004). Though important, the issue of vehicle and personnel hygiene is not critical for birds on the farm being cleared, but is so to prevent transfer to other farms (particularly at thinning) via the catching teams and vehicles (Corry and Atabay, 2001).

#### 1.13.7.5.9 Processing

Processing can be broken down in to three sections: transport, pre-chill and post chill. Transport has been identified as the major source of *Campylobacter* spp. contamination on the surface of broilers (Byrd et a., 1998), whereas during pre-chill, the process of plucking (Takahashi et al., 2006) and evisceration (Byrd et al., 1998) have been identified as main contributors.

Within transport and processing, there exist a complex set of production factors and variables which influence the levels of *Campylobacter* spp. detected (Stern and Roback, 2003). This is not just in terms of the percentage of live birds that test positive but also the number of bacteria on the carcass and subsequent pieces of chicken. Indeed, PCR analysis (Takahashi et al., 2006) has shown that contamination of chicken wing meat was mainly from farm strain carryover, contaminated during processing. Overall, populations of *Campylobacter* spp. decrease over the course of processing (Berrang and Dickens, 2000), though counts increase after plucking, before continuing the downwards trend. Interestingly Hald et al. (2000) found that whilst 52% of flocks tested positive for *Campylobacter* spp. before slaughter, by the end of processing only 24% of flocks were positive. This is in part due to *Campylobacter's* susceptibility to environmental stress (e.g. desiccation) and part due to the bacteria not reproducing outside of the gut environment (Berrang and Dickens, 2000). This decline appears to continue along the food chain, as broiler carcasses and fillets sampled at retail level were significantly less contaminated than samples from production plants (Uyttendaele et al., 2006). This shows that whilst it is vitally important to control processing, ultimately the bacteria that are present on and in the broiler on entry to a factory determine the counts on the finished product.

Within transport the key factors that affect the level of surface contamination include the length of feed withdrawal time, the method of catching/loading, and the length of transport time. Feed withdrawal has been covered previously, but work (Bolder, 2007) has shown that this typical practice increases the liquid content of the crop and thus increases the possibilities for *Campylobacter* contamination during processing. Stern et al. (1995) found significant increases in the prevalence of *Campylobacter* spp. on chicken carcasses after transport (12% prior and 56. % post). The study found that the numbers of the bacteria also increased from 2.71 $\log_{10}$ CFU per carcass to 5.15 $\log_{10}$ CFU per carcass, and concluded that transport and holding prior to processing contributes to the high levels of *Campylobacter* spp. typically isolated from poultry carcasses. This agrees with findings by others such (Nauta and Havelaar, 2007), who found higher *Campylobacter* spp. counts in transportation faeces than normal, probably as a result of increased Caecal content in the faeces due to the stress. This stress has been reported to result from live bird handling and leads to excessive faecal excretion (Bolder, 2007). These combine together to exacerbate the problem, and highlight the importance of high welfare standards during catching.

Once at the processing plant, the pre-chill phase can be thought of as the killing, scalding, plucking and evisceration, the latter two reported to be critical points on the processing line to prevent cross-contamination and therefore to control *Campylobacter* spp. (Takahashi et al., 2006). This has been highlighted by Berrang et al. (2004) who tested a range of caecal quantities (2–100 mg) on one half of a chicken carcass (other one as a negative control) and reported that just 5 mg of intestinal content cause a significant increase in the number of *Campylobacter* spp. on the carcass rinse ($P<0.01$). This is very important as vicera may rupture during evisceration, and thus spread large numbers of bacteria over multiple carcasses (Rosenquist et al., 2006). If rupture occurs, washing can decrease the number of *Campylobacter* spp. on the carcass, though this must be managed correctly (Rosenquist et al., 2006). Pertinently, a reduction in carcass *Campylobacter* spp. concentrations could also be achieved by decreasing the concentration of the bacteria in the intestines of living birds (Rosenquist et al., 2006).

On-farm controls can decrease the likelihood of problems during processing. For instance, certain illnesses may increase the incidence of gastro-intestinal ruptures (Singer et al., 2007) and thus increase the microbial contamination and cross contamination. Diseased birds may also support higher levels of pathogens such as

*Salmonella* spp. and *Campylobacter* spp. (Singer et al., 2007). Finally, contamination can be increased due to further procedures within the factory by poultry inspectors when removing diseased parts (Singer et al., 2007).

The post-chill phase of production includes the chilling process itself and the subsequent sorting, cutting up and packaging, all of which give possibilities for cross contamination between carcasses or cuts of meat.

Air chilling has been reported as a more effective means of controlling *Campylobacter* spp. numbers than water (immersion) chilling. Rosenquist et al. (2006) explains this is due to the susceptibility of *Campylobacter* spp. to desiccation. Other research has looked at the addition of chemicals to chill tanks as a method of decreasing *Campylobacter* counts and is reviewed in section 1.13.7.8.4 of this literature review.

Implementing a form of anti-microbial treatment process for poultry carcasses after evisceration but prior to further processing (for instance by the addition of chemicals to chill tank water) would seem to both be effective in reducing pathogenic bacterial risk and practical. A number of options such as organic acids, namely, lactic, acetic, citric and benzoic acids, (Kim and Marshall, 2000; Hwang and Benchit, 1995; Sakhare et al., 1999; Zeitoun and Debevere, 1990), chemicals such as chorine and hydrogen peroxide (Berrang et al., 2007; Wagenaar and Snijders, 2004) and moist temperature treatments (Avens et al., 2002; De Ledesma et al., 1996; Purnell et al., 2004). Of these, although they show potential benefits, the chemical and organic acid treatments are not currently permitted for use in the EU (Rosenquist, 2008).

#### 1.13.7.5.10 Other factors

Some authors have reported a positive correlation between *Campylobacter* spp. infections and *Salmonella* spp. in poultry (Jacobs-Reitsma et al., 1994 and 1995), though recently no association has been found (Rasschaert et al., 2007b; Wedderkopp et al., 2000). No association between the two organisms has been found in environmental samples such as ground and surface waters (Schaffter and Parriaux, 2002). The FDSCG strategy plan report (2001) perhaps explains this difference due to the effects of measures implemented to reduce the number of *Salmonella* cases from consumption of poultry products, which seems to have had very limited effects upon *Campylobacter* spp. within the same birds. Interestingly, whilst *Salmonella* spp. have been associated with

eggs (and hence vaccination for layer hens implemented), Fonseca et al. (2006) found no evidence of *Campylobacter* spp. transmission into eggs (either in the contents of on the shells) even by confirmed *Campylobacter* spp. carrying breeder hens. The study concluded that the vertical transmission of the bacteria is unlikely. Not only does this apply to chicks, but also to human cases from egg consumption. Similarly, (Jacobs-Reitsma et al., 1995) failed to isolate *Campylobacter* spp. from samples taken from a broiler hatchery.

It has been reported that in poultry, *Campylobacter* spp. colonisation reaches a plateau within the first five days after infection, of up to $10^{10}$ CFU $g^{-1}$ in caecal contents (Newell, 2002). Initially they are found throughout the bird's gastro-intestinal tract, though after a while this is confined to just the cecum and lower intestine, which persists until slaughter. However, even during this, periodic colonisation of the ileum, jejunium and duodenum (Newell, 2002) can occur, as well as the extra-intestinal infections into organs such as the liver and spleen.

Rosenquist et al. (2006) sampled the intestinal contents of six flocks at slaughter, and found a prevalence of 97-100%, with a mean count of 7.38 $log_{10}$ CFU $g^{-1}$. These results are within the same range as other published data on *Campylobacter* counts in chicken intestinal material (5–8 $log_{10}$ CFU $g^{-1}$: (Stern et al., 1995; Stern and Robach, 2003).

### 1.13.7.6 Method of Sampling

A review of sampling methods published by Corry et al. (1995) concluded that although there is no generally accepted "standard" method, there are some generally agreed procedures. For live birds these include boot sock swabbing (McCrea, 2005), intestinal contents (faecal droppings) (Rosenquist et al., 2006) and cloacal swabbing (Evans and Sayers, 2000); whilst Caecal contents (Berndtson et al., 1992), neck skin swabbing (Berndtson et al., 1992), neck skin sampling enrichment broth (Jørgensen et al., 2002), carcass cavity swabs (Berndtson et al., 1992) and carcass rinses (Harrison et al., 2001) have been conducted on factory samples and farm culls.

Typically, cloacal swabs from live birds or fresh faecal matter are used for flock testing. Evans and Sayers (2000) reported that by cloacal testing just four birds from different areas in each quarter of a broiler house (total of 16 birds/house), the maximum probability of failing to detect *Campylobacter* infection in a flock containing at least 5%

infected birds was estimated to be <3%. Collection of fresh faecal matter can also be used in flock testing for *Campylobacter* spp. (Berndtson 1996a). For enumeration purposes, caecal contents can de directly plated onto agar, thus culls or factory samples can be used to remove the paired caeca from the intestines. Some authors have noted that slaughterhouse sampled caecal contents are a more accurate method of sampling than on-farm faecal sampling as the methodology and environment is more controlled (Russa et al., 2005) and the lack of individual bird handling giving substantial labour savings.

Interestingly Skov et al., (1999) found that in terms of sensitivity for detection (for *Salmonella* spp.), overshoe or sock-swab methods (five pairs) were comparable to testing large numbers of live birds (300 – 60 pools of 5 birds) by fresh faecal samples. If this is also comparable for *Campylobacter* spp. then major savings in both time and money can be made for farmers, as the sampling can even be conducted as part of routine house checks.

Rosenquist et al. (2006) found that there was a correlation (independent of differences in scalding temperature and de-feathering) between faecal matter testing and neck skins, though the numbers of bacteria found were 4.2 $\log_{10}$ units lower on neck skins. In the UK, a study found no difference between the isolation and enumeration methods for *Campylobacter* using either neck-skin analysis (about 2.5 g neck-skin) or the carcass-rinse method plus neck-skin sample (Jørgensen et al., 2002). Boot swap swabbing can be conducted for the detection of *Campylobacter* spp. infected flocks (McCrea, 2005), as litter has been shown to reliably test positive for *C. jejuni* once infection in the birds was established (Montrose et al., 1985).

**1.13.7.7 Laboratory Methods**

The nature of the sample taken determines which laboratory methods are most suitable for analysis. For instance, environmental samples with low expected bacterial counts require more sensitive methods to determine the presence of *Campylobacter* spp. (Donnison, 2003), whereas caecal samples with higher expected values are more suited to non-enrichment direct plating onto selective agar mediums (e.g. modified charcoal cefoperazone deoxycholate agar; mCCDA), enabling colony numeration. Obtaining numbers of bacteria from samples with low levels is more difficult, with even presence/absence tests can be problematic and necessitate the use of enrichment broths

which then make numeration estimates inaccurate. Predominantly, one of three enrichment broths are used; Preston (Berndtson et al., 1992; Bolton and Robertson, 1982), Bolton (Solomon and Hoover, 2004) and Exeter (Humphrey et al., 1995), and incubated for 48 hours at 37°C (Humphrey et al., 1995: Jørgensen et al., 2002). Bolton broth has been reported to enable faster resuscitation and growth of *C. jejuni* compared to Preston broth, though the latter is selective against *E. coli* which can cause problems when trying to isolate *C. jejuni* on agar plates (Jasson et al., 2009), whilst Exeter has been reported to be more sensitive for environmental detection and naturally contaminated chicken meat (Humphrey et al., 1995).

Other methods of numeration involve the bacteria counts being regulated using peptone water (typically 1 in 10 dilution; Berndtson et al., 1992); e.g. caecal contents can be diluted 1 g:9 ml of peptone water and muscle samples 10 g:90 ml of peptone (stomached for one minute). However, if expected levels are very small numeration is sometimes impossible and therefore enrichment is necessary. For instance with Preston broth (1 g muscle to 5 ml of broth; Berndtson et al., 1992). These liquid fractions can then be plated onto selective agar medias such as mCCDA, Preston selective agar (Bolton and Robertson, 1982) or Columbia Blood Agar (Oxoid, 2010b), modified Abeyta-Hunt-Bark (mAHB) (Rosenquist et al., 2006) (after incubation in the case of the enrichment process) and incubated microaerophilically at 42-43°C for 24 h. As *Campylobacter* spp. are theromophilic, these high temperatures form part of the selective process, restricting the growth of many other species of bacteria.

Other methods of testing involve swab samples. Neck skins and the peritoneal cavity can both be sampled by rubbing a cotton swab over a 2 × 2 cm area, placed into a tube containing 9 ml of peptone water (Berndtson et al., 1992). If enrichment is necessary (for the reasons described previously) then 5 ml of Preston broth may be substituted for the peptone water. Others such as Rosenquist et al., (2006) tested neck skins by adding a 10 g sample to 10 ml of Buffered Peptone Water (BPW), then producing a series of 10-fold dilutions with BPW. These were then plated out (100 μl) onto mAHB agar plates, and incubated micro-aerobically for 48 h at 42°C.

Finally, Preston selective agar can be used for bacteria numeration (Bolton and Robertson, 1982), which consists of *Campylobacter* agar base (Oxoid CM689), 5% (v/v) saponin-lysed horse blood together with *Campylobacter* selective supplement

(Oxoid SRl 17) (Oxoid, 2010c). For the purpose of quantitative analyses, the agar content may be increased to 2% to limit the spread of *Campylobacter* spp. colonies.

Other authors such as Scherer et al. (2006) have compared the most probable numbers (MPN) method to direct plating onto agar for numeration. They report that MPN to be unsuitable for the enumeration of *Campylobacter* spp. in chicken skin samples as the formation of a layer on top of the liquid media leads to unreliable MPN results.

Both enrichment and numeration methods produce presumptive colonies of *Campylobacter* spp.. Confirmation techniques may potentially involve a number of stages such as latex agglutination (Oxoid, 2010d), gram-staining and examination for motility (Fernandez et al., 2000). This latter involves suspending a droplet of liquid containing bacteria taken from the presumptive colonies on a small glass cover slip. This in turn rests a few millimetres above a full size slide. By using microscopic visualisation (Fernandez et al., 2000) the bacteria can be checked for their characteristic corkscrew movements. Further confirmation tests involve sub-culturing presumptive colonies (for instance onto horse blood agar plates) and incubation both aerobically and microaerophilically (to confirm the bacteria only grows in the latter conditions) and subsequently testing for oxidase and catalase activity. Positive isolates are considered to be *C. jejuni or C.coli* (Berndtson et al., 1992).

### 1.13.7.8 *Campylobacter* spp. control measures

In view of all of this, much research has been conducted in order to try and control the transmission and prevent colonisation of *Campylobacter* spp.. Whilst some authors (de Zoete et al., 2007) have suggested that the vaccination of chickens against *Campylobacter* is the solution, at present no vaccine is available. Others such as Kitchen and Vaughn (2007) discuss efforts to produce a human vaccine, particularly favoured by the US military. Other measures being investigated or promoted include strict biosecurity, changes in management practices such as thinning, the addition of chemicals to drinking water and post slaughter carcass treatments.

Indeed in Europe, bodies such as the EFSA propose to divide the problem into two parts. Firstly, to reduce the proportion of positive flocks by "*the application of strict biosecurity measures in primary production including the use of non-contaminated drinking water*"; and secondly, to reduce the numbers of bacteria on the carcasses by

"*reducing faecal spread during slaughter and further processing and by using appropriate physical or chemical decontamination techniques where permitted*" (EFSA, 2004).

**1.13.7.8.1 Feed**

Although feed has been reported not to be a causal factor for *Campylobacter* spp. colonisation (Jacobs-Reitsma et al., 1995), research has focused on a variety of feed supplements given to broilers containing additives such as; leek extract (Lee et al., 2004) – reported to be effective at 2.0 mg $ml^{-1}$, and algal meal (Waldenstadt et al., 2003), which had no effect on *C. jejuni* though did show a reduction in *Clostridium perfringens*. Others have investigated fermented liquid feeds (Heres et al., 2003, 2004) containing high levels of lactic and acetic acids, which were reported to delay flock colonisation although not reduce within-flock transmission after a *Campylobacter* spp. infection was introduced into a broiler house (plausibly this shows that acids are effective at low levels of bacterial challenge but not at the higher levels found in colonised faecal matter.)

**1.13.7.8.2 Competitive Exclusion cultures**

A number of authors have discussed the possible benefits of competitive exclusion (C.E.) cultures (Stern et al., 2001), the theory being that lactic-acid producing bacteria out-compete pathogens such as *Campylobacter*, *listeria*, *C. perfringens* and *Salmonella* (Schneitz, 2005) for nutrients, and have colonised the gastro-intestinal tract prior to the point of pathogen challenge. However, it has been reported that the level of protection against pathogens is not great, and if used solely, would not merit the effort and financial outlay involved (Stern et al., 2001). Similarly Aho and Hirn (1988) discuss how although long-term C.E. culture use in Finland has contributed to the low incidence levels found there, they are unsure of its true effectiveness, as other factors may be the cause of this, such as the small-scale of the industry and above all, the cold climate.

**1.13.7.8.3 Phages**

Phages have also been suggested as another potential control solution to combat *Campylobacter* spp. infection (Attebury 2003a, 2003b), by the addition of suitable phages during carcass processing. This is not without its limitations however, as certain methods of chilling have been shown to remove the majority of *C. jejuni* phages from retail chicken meat (Tsuei et al., 2007).

#### 1.13.7.8.4 Post-Slaughter implementations

A great deal of research has been conducted into control methods that can be employed in a processing plant post-slaughter. As mentioned previously, *Campylobacter* spp. only reproduces within the gastro-intestinal tract of a host animal, and thus after the evisceration process, numbers of the bacteria on a carcass only decrease along the processing line (Berrang and Dickens, 2000). Interestingly, others confirm this, e.g. Hald et al., (2000) who found that whilst 52% flocks tested positive prior to slaughter, when tested at the end of processing this figure had more than halved.

*Campylobacter* spp. are predominantly found on the surface of birds and carcasses (Whyte et al., 2006), at high levels of prevalence (>70%: Scherer et al., 2006), whilst other studies have reportedly failed to isolate the bacteria in muscle samples (Altmeyer et al., 1985). When these facts are considered together, they support the theory that any surface intervention post-evisceration could be very effective as carcasses/meat portions would be highly unlikely to be re-contaminated by *Campylobacter* spp. (EFSA, 2004). However, it must be borne in mind that *Campylobacter* spp. adhere to poultry skin, forming a biofilm, and can be entrapped in ridges and crevices of the skin (Corry and Atabay, 2001). It has also been discussed how during scalding feather follicles open, providing the opportunity for the ingress of the bacteria (Berndtson et al., 1992). Following this, during chilling, the follicles close once more, trapping the bacteria inside. It was therefore theorised by the authors that the effects of a carcass disinfectant wash or spray would be limited, as it is unlikely to penetrate all of the feather follicles (Berndtson et al., 1992).

However, a number of chemical surface treatments have been investigated and found to be effective in reducing levels and the prevalence of *Campylobacter* spp. in chickens (e.g., Avens et al., 2002; Berrang et al., 2007; Whyte et al., 2003). For instance the use of chlorine as a disinfectant has been investigated; and though simply washing a carcass pre-eviceration with chlorinated water was shown to have no effect, chlorinated chill tanks were found to be effective (Berrang et al., 2007), though not by Berndtson et al. (1996a) and the same chemical added to a carcass-wash prior to evisceration did not have a significant effect (Berrang et al., 2007). The European Union is in the process of preparing a list of chemical decontaminators, though as yet none are permitted for use (Rosenquist, 2008).

Moist heat treatments are permitted however and have been proven to be effective using both steam at >90°C (Avens et al., 2002; James et al., 2007; Whyte et al., 2003) and hot water washes >80°C (Avens et al., 2002; Corry et al., 2007;). These do have problems however as as the length of time needed to be effective changes the appearance of a carcass (Avens et al., 2002; Whyte et al., 2003), making it visually unacceptable to consumers – the skin shrinks and changes colour (James et al., 2007). It is important to note that this surface cooking is not the sub-cutaneous meat and this is therefore not a problem if the birds are for processing and not whole-bird sale (Avens et al., 2002).

Whilst other meats (e.g. skinned beef carcasses) can be treated effectively in this way for very short time periods such as 6 to 8 seconds (Nutsch et al., 1997), poultry carcasses are different as the skin is naturally contaminated from growing and processing, and both the texture and follicles (Avens et al., 2002) provide areas that aid *Campylobacter*'s survival for these short duration heat treatments, necessitating the length of time mentioned above that results in changes in carcass appearance. Interestingly though, James et al. (2007) tried short duration (20 s) steam treatments (80°C), followed by crust freezing, which reduced numbers by 2.9 CFU $cm^{-2}$ and didn't degrade the visual appearance of the carcass.

Irradiation has also been discussed as a possible treatment for carcasses and poultry products (Cabo-verde et al., 2004; Corry and Atabay, 2001). Studies have shown effective decontamination can be undertaken by ultraviolet radiation (Wallner-Pendleton et al., 1994) or gamma radiation (Lewis et al., 2002). However, it would appear that this technique hasn't been adopted by the broiler processing industry, plausibly due to the expense incurred, and one may imagine the negative public perception of irradiated meat.

Finally, freezing seems to work effectively without effecting carcass quality, although this does not kill every *Campylobacter* spp. on a carcass but leads to approximately a 1.5 $log_{10}$ CFU reduction in numbers (Rosenquist et al., 2006). Freezing is implemented as a control method in Denmark, Iceland and Norway, where flocks which test positive for *Campylobacter* spp. are allocated to the production of frozen chicken meat (Barrios et al., 2006; Rosenquist et al., 2006) in order to lessen the risk to consumers. However, not all consumers wish to purchase a frozen chicken, which means problems for

producers, particularly in the UK where consumers purchase nearly three times the value of fresh poultry than that of frozen (DEFRA, 2009). Whichever treatment is employed it is vital that the processing methods are optimised to ensure the minimisation of contamination from gastro-intestinal contents and cross-contamination from apparatus (Berndtson et al., 1996a; EFSA, 2004).

This therefore leaves temperature treatments as one of the few options open to processors, which although effective, can give problems with the visual appearance of the retail meat. Freezing birds would seem to be an obvious technique to reduce the risks to consumers, as conducted in some Scandinavian countries to decrease levels of *Campylobacter* spp.. However, consumers in the UK seem to have a preference for fresh poultry as opposed to frozen. The value of fresh poultry sold in the UK is 2.8 times that of frozen (DEFRA, 2009) hence there would implications for the retailing of frozen chicken. Similarly, whilst removal of the skin has been shown to decrease the level of *Campylobacter* spp. on chicken meat (FSA, 2009), some consumers inevitably prefer to buy skin-on chicken.

**1.13.7.8.5 Hygiene Issues**

Good hygiene has thus been purported to be the panacea for controlling *Campylobacter* spp. (Arsenault et al., 2007; Allen et al., 2008c; Berndtson et al., 1996a; EFSA, 2004; Gibbens et al., 2001; Van de Giessen et al., 1996).

Whilst exemplary practices in hygiene of the breeder flock, egg handling and hatchery are vital for disease control in general, especially for bacteria such as *Salmonella* spp., the same does not apply with *Campylobacter* spp. (Evans and Sayers, 2000). This is because the bacterium is not transmitted into the yolk sack from the parent bird, nor does it survive on the arid surface of the egg shell for long periods (Fonseca et al., 2006).

However, whilst vertical transmission is unlikely by these means, it potentially can be carried over from previous flocks if correct hygiene practices are not followed (Evans and Sayers, 2000). Broiler crops typically have short turn-around times between them, and the cleaning methods in this period involve high levels of water usage, providing high-humidity conditions that promote *Campylobacter* spp. survival. These combine to increase the likelihood of carry-over between flocks. Nevertheless, some doubts remain as to the importance of flock carry-over as a major factor of *Campylobacter* spp.

infection in broiler flocks (EFSA, 2004). Whilst some studies have reported the issue to be important (Petersen and Wedderkopp, 2001) others have found no evidence to support this, despite highlighting it as a possibility (Berndtson et al., 1996b; Evans and Sayers, 2000).

Whilst studies have shown (Gregory et al., 1997) that the time of introduction and rate of colonisation is not effected by the newness of a house, or whether or not it had housed previous flocks of broilers, this could be due simply to the correct sanitation procedures being followed. Conversely, other studies have shown that the building being in a good state of repair had a correlation with an increase in the number of days to infection (Evans and Sayers, 2000). The same study also found other factors had similar effects such as the effectiveness of sanitation methods prior to chick placement, sanitation of the drinking water system and more frequent boot-dip changes, though the authors also discuss how these could be interlinked-indicators of general good hygiene practice rather than stand-alone factors.

**1.13.7.8.6 Litter**

Whilst the complete removal of the previous broiler crop's litter is vital to a *Campylobacter* spp. free status of the subsequent flock (Evans and Sayers, 2000; Kazwala et al., 1992; Montrose et al., 1985), good management of the litter is important too. Not only is good quality litter important for welfare standards (RSPCA, 2008), but poor management leading to wet litter gives more favourable conditions for *Campylobacter* spp. survival (Berndtson et al., 1996b) and thus increases the probability for flock infection.

Having said this, even well managed litter does not prevent flock colonisation as even just one colonised broiler typically leads to rapid infection of the rest of the flock within a short space of time (Evans and Sayers, 2000; Hald et al., 2001; Jacobs-Reitsma et al. 1995).

**1.13.7.8.7 Cross-contamination**

As discussed previously, *Campylobacter* spp. contamination from external sources can occur in a variety of ways and so too can cross-contamination between two flocks on the same farm in a number of ways. The primary source is via farm workers as their hands, clothing and especially footwear are all methods of *Campylobacter* spp. transfer

described by many authors (Evans and Sayers, 2000; Gibbens et al., 2001; Kapperud et al., 1993; Refrégier-Petton et al., 2001). Indeed, Gibbens et al., (2001) goes as far as to suggest a set of dedicated boots and overalls for each house, together with multiple boot dips and hand sanitisers. Furthermore, this study found that by the strict implementation of these measures the risk of infection was reduced by 50%. This would only be as effective as the workers' attitude to, and knowledge of, biosecurity; as described by many studies (Berndtson et al., 1996a; Evans and Sayers, 2000; Refrégier-Petton et al., 2001).

In addition to rodents and insects being a source of *Salmonella* spp. infections (Henzler and Opitz, 1992; Heres et al., 2003; Meerburg et al., 2006; Meerburg and Kijlstra, 2007), insects (Berndtson et al., 1996a; Arsenault et al., 2007) and rodents (Arsenault et al., 2007) have also been identified as a methods of both introduction and transmission between houses. Indeed Berndtson found the same serotype of *Campylobacter* spp. in both the insects and chicken flock. The link between poultry and insects is well established (Shane et al., 1985), with studies showing that contaminated poultry infected previously negative houseflies *(Musca dornestica)* in laboratory conditions, and when these contaminated flies were transferred to specific-pathogen-free chickens, these birds too became infected. When applied to the on-farm situation, this backs up Hald et al.'s findings (2007) that the incidence of *Campylobacter* spp. positive flocks in summer decreased from 45% to 11% with the provision of fly screens.

This illustrates the challenges faced by broiler producers in achieving strict bio-security conditions. However, whilst Hald et al. (2007) demonstrate that measures to limit the movement of insects into and out of broiler sheds are effective in reducing the incidence of positive broiler flocks, the measures have not been widely implemented by the UK broiler industry. Plausibly, this could be due to a combination of lack of awareness of the importance of insects as a vector of *Campylobacter* spp., financial effect of the low profitability per bird, and lastly, the lack of legal requirement to reduce the prevalence of *Campylobacter* spp. in broilers.

Other hygiene issues include those surrounding transportation. For instance the crates have been found to be positive for the presence of *Campylobacter* spp. (Allen et al., 2008b), illustrating that factory cleaning methods were inadequate for sanitizing the crates. Other studies have shown contaminated crates to have the ability to infect

previously negative birds (Slader et al., 2002). This was suggested to be due to the ineffective cleaning regimes in the factory, and the high frequency of use. As mentioned previously, Allen et al., (2008a and b) demonstrated that the combined use of ultrasound and hot water immersion (>60°C for 3 to 6 minutes) to be significantly more effective ($P<0.05$) than current pressure washing techniques.

Finally, the hygiene of the bird's production inputs has been examined, namely feed and drinking water. Feed has been tested and discounted as a route of infection for *Campylobacter* spp. (Jacobs-Reitsma et al., 1995; Montrose et al., 1985) unlike *Salmonella* spp. (Cox et al., 1983; Van Immerseel et al., 2006; Veldman et al., 1995), suggested being due to *Campylobacter's* susceptibility to desiccation (Bell, 2006).

Whilst municipal, chlorinated water supplies are unlikely to be a primary source of *Campylobacter* (Evans and Sayers, 2000), untreated drinking water has been identified as a possible route of infection (Arsenault et al., 2007; EFSA, 2004; Kapperud et al., 1993). Linked to this is the hygiene of the drinkers, as without a high standard of cleaning and disinfection, they have been identified as being responsible for transmitting the bacteria within a flock (Evans and Sayers, 2000). The same author also discusses the design of these drinkers and how individual drinkers may be better than communal drinkers as there are fewer opportunities for the spread of *Campylobacter* spp. Drinkers are typically contaminated with a combination of faeces, feed and bedding, which when combined with high levels of moisture, gives rise to a situation that is conducive to the survival of pathogenic bacteria (Chaveerach et al., 2002), which is particularly important given the ease with which birds can be contaminated from drinking out of these vessels. Indeed, studies of using a suite of sanitation techniques which also included cleaning and disinfecting water pipes and chlorinating the drinking water, reported a reduction in the colonisation of broiler houses by *Campylobacter* spp. from 81% to 7% (Pearson et al., 1987, 1993).

## 1.14 Organic Acid Treatments

Studies of organic acids on *Campylobacter* spp. have shown both *in vitro* and *in vivo* effects (Byrd et al., 2001; Van Deun et al., 2008). However, other studies such as Chaveerach et al. (2004) found no preventative effects upon colonisation by adding organic acids to the birds' drinking water, and almost all of the birds tested became colonised. Some authors have suggested these inconsistent results are as a result of

uncontrolled variables within trials, for example the buffering capacity of dietary ingredients (Dibner and Buttin, 2002). It has been discussed how the effects of organic acids are pH dependant (Chaveerach et al., 2004).
A complementary technique to improved hygiene is the use of organic acids within both drinking water and feed to decrease colonisation of the gastro-intestinal tract by pathogenic bacteria such as *Salmonella* spp., (Van Immerseel et al., 2006) without damaging intestinal epithelial cells (Chaveerach et al., 2004). In turn this has the possibility of decreasing contamination of both carcasses and eggs (Van Immerseel et al., 2006). Interestingly, both acids and their salts have been shown have anti-bacterial effects (Dibner and Buttin, 2002).

Organic acids have been shown to work against bacteria such as *Campylobacter* spp. and *Salmonella* spp., via the undissociated acid molecule being diffused across the cell membrane, where the pH change in the cell's cytoplasm releases the hydrogen ion that in turn decreases the pH of the cell cytoplasm (Baik et al., 1996; Chaveerach et al., 2002; Ricke 2003). This in itself disrupts the intracellular reactions such as enzymes and in addition the cell uses energy to expel the free hydrogen ion, resulting in the depletion of cellular energy and in turn leading to bacteriostasis (Dibner and Buttin, 2002; Ricke, 2003).

Therefore the antimicrobial activity of any organic acid given to poultry will be dependent on the pH of the gastro-intestinal tract contents (Dibner and Buttin, 2002), reported to be strongest against *Campylobacter* spp. at pH 4.0 (Chaveerach et al., 2002). In weaker acid conditions above pH 4.0 *Campylobacter* spp. has been reported to survive and suffer from only a slight decrease in culturability (Chaveerach et al., 2002).

Interestingly not all organic acids have been reported to have equal antibacterial effects. For instance, a small excess of undissociated formic acid has been shown to be more effective against *Campylobacter jejuni* than a large excess of undissociated acetic or propionic acids at the same pH *in vitro* (Chaveerach et al., 2002).

Whist some studies have shown that a combination of formic and propionic acids can reduce the incidence of *Salmonella* spp. colonising the lower gastro-intestinal tract of birds (Hinton and Linton, 1988) others have failed to show that this blend prevents

colonisation of *Salmonella* spp. in live birds (Oliveira et al., 2000; Thompson and Hinton, 1997).

## 1.15 Other antibacterial treatments

A number of studies have investigated methods to decrease the bacterial load of chickens and chicken meat to improve hygiene and decrease the risk for consumers. These have been conducted in a variety of applied (e.g. addition of feed supplements) and theoretical (e.g. modelling of pathogen flows) means, focusing on different points within the production process.

### 1.15.1 Transport crate hygiene

The hygiene of transport crates is important in the spread of pathogens such as *Salmonella* and *Campylobacter* between chickens (Slader et al., 2002). Allen et al. (2008a and b) demonstrated that the use of ultrasound in combination with hot water immersion (>60°C for 3–6 minutes) to be effective in reducing numbers of both *Campylobacter* spp. and Enterobacteriaceae (4 $\log_{10}$ unit reduction), when compared to current procedures such as pressure washing. Hot water immersion in combination with brushing, hot washing and subsequent chemical disinfection was also shown to be effective in Allen et al. (2008b).

As skin and carcass surfaces contain a large proportion of potentially pathogenic load, reducing the bacterial population on the surface of birds both pre- and post-slaughter may lead to significant gains in the microbial quality of the meat (Avens et al., 2002). A number of studies have attempted to decrease the number of bacteria upon processed carcasses by using water at elevated temperatures (Avens et al., 2002; De Ledsma et al., 1996; Purnell et al., 2004). Surface treatments have typically been implemented after the completion of evisceration and carcass washing prior to chilling (Purnell et al., 2004). De Ledsma et al. (1996) applied water at 95°C for 5 s; whilst Avens et al. (2002) applied for >3 min; and Purnell et al. (2004) applied water at 75°C for 30 s3). However, this latter experiment was found to detrimentally effect the carcass quality as the skin often broke during packaging. A subsequent trial of 70°C for 40 seconds did not have this problem, and yet still gave a >1 $\log_{10}$ unit reduction for both aerobic plate counts and Enterobacteriaceae (though not *Campylobacter* numbers). The higher temperatures in Avens et al. (2002) gave greater reductions (3 $\log_{10}$ units) though still did not sanitise the carcass completely, which means there is still the possibility of pathogens such as

*Salmonella, Campylobacter, Listeria moncytogenes* and *E. coli* O157:H7 being on the carcass in infective doses (Avens et al., 2002). Furthermore, it is worth noting that the detrimental effects of temperature on the carcass skin is only really problem for those carcasses destined for sale as whole bird, and not for a large proportion of birds which are processed into value added portions (Avens et al., 2002). This would thus help to sanitise the skin which would in turn reduce the cross-contamination of bacteria onto the sub-cutaneous meat.

De Ledsma et al. (1996) compared hot water treatments (95°C, 5 seconds) with a 10% tri-sodium phosphate dip prior to the same hot water treatment and a negative control and found both of the hot water treatments to be significantly ($P<0.05$) more effective in reducing the counts of a number of pathogenic bacteria (*S. typhimurium*, *S. aurus*, and *L. monocytogenes*) than the negative control treatment. Interestingly, although the tri-sodium phosphate dip was more effective than just hot water against the *Salmonella* spp., it was not as effective against both *S. aurus* and *L. monocytogenes*.

Other chemicals have been studied as possible surface decontaminates of poultry meat. Hwang and Benchit (1995) found a 0.5% lactic acid/0.5% sodium benzoate solution to significantly reduce *Salmonella* spp., *C. jejuni*, *S. aurus*, *L. monocytogenes* and *E. coli* O157:H7. This effect was observed on chicken wings both immediately after treatment and after storage of up to 8 days at 4°C. Multiple log reductions were found for *Salmonella*, *C. jejuni* and *E. coli* O157:H7, with less of an effect for *S. aureus*. *Listeria* number decreased slightly in the treatment, though the control actually increased during storage. Thus the study concluded that the lactic acid/sodium benzoate solution retarded the growth of all species, therefore extended the shelf life of the chicken wings and enhanced safety.

Lactic acid was also used by Kim and Marshall (2000) who investigated its effects, in addition to those of acetic and citric (all 1% solution) on aerobic plate counts. They found that dipping wings in these acids for 10 minutes significantly ($P<0.05$) decreased the plate counts for up to 12 days. Acetic acid was found to give best results (approximately a 5 $\log_{10}$ reduction at 12 days), with citric and lactic acids giving similar results of around 3 $\log_{10}$ reduction at 12 days compared to a control treatment.

Acetic and lactic acids were also used by Sakhare et al. (1999) who washed carcasses with water containing either acid at every step of processing. Acid treatment by dipping carcasses into either 0.5% acetic or 0.25% lactic solutions was found to be effective, as was the addition acids to scald tank waters, when testing for TVCs, *S. aureus*, and coliforms. Zeitoun and Debevera (1990) also used lactic acid, and found buffering the acid gave lower skin pHs and increased chicken leg shelf life from 6 days to 12 days at 6°C. Wagenaar and Snijders (2004) used 3% hydrogen peroxide solution in water to decontaminate carcasses via immersion dipping and showed that this significantly reduced ($P<0.01$) the counts of Enterobacteriaceae and TVCs at 3.5 hours, 24 hours and 7 days of storage at 1°C.

**1.16 Lime Treatment of Blood**

Commercial poultry processing produces a number of wastes, most problematic of which is the animal's blood, due to the high volumes generated (15 million tonnes per year in the EU; Sharrock et al., 2009) and very high pollutant load (Del Hoyo et al., 2008). This has a legal requirement (EU Animal By-Product Regulations, 1774/2002) to be disposed of in a safe manner (Anon, 2005). This legislation outlawed the spreading of untreated abattoir blood waste onto agricultural land as a method of disposal, stating that the waste must be rendered or incinerated to be considered safe.

Rendering is the heating of animal products (either in a dry or moist condition) in order to kill harmful bacteria and obtain useful products (Mittal, 2006). For blood this consists of three stages: sterilization, drying and the separation of the protein and fat (Krenk, 1991). Dried poultry blood is typically 95% protein (Salminen and Rintala, 2002a). The heating process (115-230°C for 45-85 minutes, Kiepper, 2003) breaks open the tissue cells which releases the fat molecules and allows to be separated at a later stage (Kiepper, 2003). These products can be used as components in feed for pets and utilized in many industrial processes (Anderson, 2006).

Rendering is highly effective in minimising the risks to human health from bacteria such as *Campylobacter*, *Escherichia coli, Listeria, Salmonella, Bacillus anthracis, C. botulinum, Leptospisa, Mycobacterium tuberculosis and Yersinia* (Woodgate, 2006).

Whilst rendering is the heat treatment of animal products, incineration is the burning of such matter. Poultry carcasses burn at 300°C with the emission of smoke, whilst at

500°C the ashes still have lots of organic matter left in them, though increasing the temperature further to 750°C leaves off-white ash (Sharrock et al., 2009). Flame temperatures of nearly 1000°C are required for industrial destruction of meat and bone meal, of which blood wastes may make up a small percentage (Sharrock et al., 2009). Incinerated wastes produce gasses, steam, and ash as their outputs, with the latter having the potential to be used as fertilizer in some circumstances, e.g. duck carcass ash as phosphate fertilizer where legally permitted (Sharrock et al., 2009).

Incineration is similar to rendering in that it has low overall risks to human health, but gives rise to moderate risks human health from particulates ($SO_2$, $NO_2$), dioxins and radiation (Woodgate, 2006). As a result incinerators are required to adhere to strict environmental pollution limits (Lee and Huffmann, 1996).

The increasing costs associated with the process of incineration make this a less attractive option for the disposal of blood waste (Sharrock et al., 2009). In addition, the nature of incineration means that dry matter is preferred for the process, meaning blood waste on its own is less attractive as it has a high heat requirement (hence high cost) in order to decrease the moisture content prior to clean burning at high temperatures (Boost and Poon, 1998).

Compounded with this, a reported decline in demand for rendered products has resulted in the closure of some rendering plants (Mittal, 2006). The remaining plants charge poultry producers a fee for the disposal of their blood, whereas previously they would take it for free (Mittal, 2006). Thus, there is interest in other techniques for poultry blood disposal, particularly outside of the EU, where these are already permitted.

Poultry blood waste can be treated using a variety of composting techniques (Bicudo and Goyal, 2003; Mittal, 2006), ranging from open-air windrows and piles, to self-contained vessels, and anaerobic digesters (Salminen and Rintala, 2002a; Salminen and Rintala, 2002b). These tend to have blood as just one constituent (e.g. 15.8%), with proportioned other material being feathers (10.5%), offal (31.6%) and bone/trimmings (42.1%) (Salminen and Rintala, 2002b).

Similarly, blood can be preserved for future nutrient recovery by lactic acid fermentation (Cai et al., 1994a, 1994b). Unfortunately though, composting takes weeks

if not months to complete, and requires constant management to optimise moisture and temperature conditions within the material, plus it requires considerable financing, suitable land plus planning approval from local authorities (Stentiford, 1996; Mittal, 2006).

Another possible blood treatment which is effective, though has limitations, is treatment via wastewater treatment plants (Bicudo and Goyal, 2003). Whilst this is used in some areas of the world, e.g. Onterio and Quebec provinces in Canada where abattoirs pay a surcharge to discharge blood into municipal sewers (Mittal, 2006), this method is both expensive and energy demanding.

Thus, even outside the EU, there is a demand for an alternative method of disposal, which prior to the 2002 regulations being introduced in the EU, land application has previously fulfilled. This prohibition has been implemented because of the potential to spread pathogens via land application, illustrated by work such as Jones (1999), both into the soil and groundwater (Wong and Selvam, 2009).

Blood is category 3 material, the lowest classification of hazardous waste from a slaughterhouse (Anon, 2005; Woodgate, 2006) – which is defined as "*animal remnants originating from abattoir processing of human food grade certified live animals*" (Sharrock et al., 2009). Some studies have failed to isolate pathogenic bacteria from it (Buncic et al., 2002). This is plausibly due to levels being lower than the test's detection limits and due to the dilution effects of the vast number of individuals (each one giving 40 g of blood; Salminen and Rintala, 2002a) processed masking a handful of positive birds (Buncic et al., 2002).

Having said this, *E. coli* O157:H7 has been detected in poultry (Akkaya et al., 2006; Schouten et al., 2005), in both layer and broiler flocks (0.5% and 1.7% respectively; Schouten et al., 2005), and blood may provide suitable conditions for the growth of pathogens. Even if initial levels are low, contamination with gut or faecal matter would introduce these pathogens into this high-nutrient growth medium and the mixing of digestive tract sludge with blood is a regular practice at some poultry-only slaughterhouses (Buncic et al., 2002). Storing the wastes for a period of time is unlikely to decrease the risk as *E. coli* O157:H7 has been recovered at high levels after 2 months of storage (Avery et al., 2005).

If land is contaminated with pathogenic bacteria, some such as *E. coli* O157:H7 can survive on grassland for up to 6 weeks (Avery et al., 2004) and in the soil for more than 5 weeks (Avery et al., 2004; Williams et al., 2007). Other work has shown the persistence of *E. coli* O157:H7 on farm surfaces such as galvanised steel and wood for a week or more given the moist, cool climatic conditions (Williams et al. 2005), showing the potential for untreated spilt blood to contaminate field gates and fences giving the potential to infect humans via hand to mouth transfer.

Pathogens such as *E. coli* O157:H7 and *Salmonella* Spp. have optimum survival rates between pH 6 and 7. Thus, raising the pH of blood with treatments such as lime (both calcium oxide and hydroxide) has been shown to give 8 $\log_{10}$ reductions in *E. coli* and *S. enteritidis* after just 15 seconds at pH 12 (Mendonca et al., 1994). This is attributed to the high pH disrupting the cytoplasmic membrane of gram-negative food-borne pathogens. Similar results have been reported by Bean et al. (2007) which showed a 7 $\log_{10}$ reduction in both *Salmonella* and coliform numbers after 2 hours at pH 12. Additionally, no *adenovirus* or rotaviruses were detected, and *Giardia lamblia* cysts were inactivated.

Whilst no work has been published on treatment of poultry blood waste with lime, lime has been shown to be effective in treating other waste products such as sewage sludges. For instance Wong and Selvam (2009) found *Salmonella*, coliforms and *E. coli* "*completely devitalised*" after 8 days at pH 12 (adding 4% CaO to anaerobically digested sewage sludge). Coliforms are used to indicate the presence of a variety of pathogens in biosolids (Qi et al., 2004) thus give an indication of the wide variety of bacteria that lime treatments may eradicate. This is also confirmed by Boost and Poon (1998) who found that 5% Calcium Oxide (CaO) mixed with 35% fuel ash and 60% sewage), prevented a variety of enteric pathogens from growing. These included *Aeromonas hydrophilia, Clostridium perfringens, E. coli, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Vibrio parahaemolyticus* and *Yersinia enterocolitica*. The authors also mention how their results suggest the minimum effective pH for killing intestinal pathogens is pH 11.

Lime has been shown to be an effective treatment for animal wastes (Duffy, 2003) plus a variety of other wastes such as human faeces in dry toilets using CaO (Niwagaba et al.,

2009), and sewage sludges using 1% Calcium Hydroxide ($Ca(OH)_2$) which killed *S. typhimurium* within 1 hour at > pH 12 (Placha et al., 2008; Plachy et al., 1996).

$Ca(OH)_2$ has also been used to improve poultry litter quality (Stringham et al., 2000) as has CaO (Ruiz et al., 2008) and not only has it been shown to have an anti-bacterial effect (Ruiz et al., 2008) but also it controls insect larvae such as beetle and housefly (Stringham et al., 2000), both of which are possible vectors of gram-negative pathogens (Evans and Sayers, 2000). Maguire et al., (2006) also used lime to treat animal waste, namely >10% CaO to treat poultry manure, and state that CaO is preferred over $Ca(OH)_2$ as it is a less bulky material and lower application rates are needed to achieve the same reduction in bacteria.

CaO has also been shown to eradicate *E. coli* O157:H7 from abattoir wastes at an application rate of 1% by Avery et al., (2009), who detected no trace of the bacteria after treatment, even though laboratory enrichment was undertaken. CaO's anti-bacterial properties have been compared to magnesium oxide (MgO) and zinc oxide (ZnO) by Sawai (2003), who found it to be the most effective against *E. coli* (followed by MgO and then ZnO), although ZnO was the most effective against *Staphylococcus aureus*.

Animal wastes may be approved by the Environment Agency for spreading onto agricultural land in the UK if they are shown to give "*agricultural benefit*" and are safe to use, causing no pollution or harmful effects to health (Anon, 2008). A number of studies have shown that blood is an effective fertilizer for agricultural land (Abbas and Choudhary, 1995; Petroczki, 2004) by injecting into the soil (Abbas and Choudhary, 1995), typically 5 cm below the surface (Chadwick and Laws, 2002).

Similarly, lime has long been used by farmers to increase soil pH, which improves fertility by increasing the soil's nutrient availability, microbial activity and cation exchange capacity (Brady and Weil, 1999). Typically this has been in the form of powdered limestone, though CaO or $Ca(OH)_2$ are used when rapid changes of soil pH are required (Brady and Weil, 1999).

Caution must be exercised when applying waste treated with CaO to land. CaO (which typically changes to $Ca(OH)_2$ on contact with $H_2O$ in the soil) can give very high,

localized, soil pH (> pH 12) even when mixed with bio-solids (sludges) (Smith et al. (1998), which may persist in the soil for weeks or more and adversely affect plant growth (Smith et al. 1998). Never the less, lime as a blood treatment would seem to have potential for the treatment of abattoir waste blood as it has both antibacterial properties and can give increases in fertility to agricultural land.

In view of all of this, it would seem that as lime treatments have been shown to be effective in combating pathogens in liquid wastes such as slurry, then lime has the potential to be effective in poultry blood waste. This is particularly relevant as many of the pathogens that have been shown to be successfully controlled by lime in these wastes have the potential to be present in blood waste. Furthermore, as both blood and lime have been shown to provide agricultural benefit when applied to farmland, the combination of both would seem to have the potential to do likewise.

# Chapter 2

## Knowledge gap assessment amongst employees of one UK broiler integrator regarding the food-borne pathogen *Campylobacter*

## 2.1 Abstract

General food-safety knowledge amongst food industry workers has been shown to have important limitations. Lack of understanding can result in poor hygiene practices, which combined with the a low infectious dose for *Campylobacter* spp., pose serious health risks to consumers. This study identified critical knowledge gaps in broiler industry workers (farm, factory and office based). Farm based employees had significantly higher levels of knowledge than factory based employees. Critical food hygiene aspects were known for *Campylobacter* spp. such as cross-contamination, the importance of hand-washing and footwear hygiene. However, certain management practices, such as thinning, were incorrectly not considered critical by farm workers who have an important role to play in the effective implementation of hygiene standards.

## 2.2 Introduction

*Campylobacter* spp. is the leading food-borne bacterial cause of gastroenteritis in the UK (Evans and Sayers, 2000) and is a major concern for poultry production systems as well as for food safety and policy (FSA, 2008). Clinical symptoms of *Campylobacter* spp. infection include abdominal cramps (Mellits et al., 2002), watery or bloody diarrhoea (MacCallum et al., 2005; Newell, 2002) and fever (Butzler, 1982). Typically the disease is self-limiting, and only occasionally with complications such as Guillian-Barre syndrome (EFSA, 2004; Kitchen and Vaughn, 2007; Mellits et al., 2002; Nauta and Havelaar, 2007).

Consumption of chicken is one of the foremost routes of human infection (EFSA, 2004; Ghafir et al., 2007), Consequently, concerted efforts from the poultry production industry have been implemented in an attempt to reduce the organism prevalence rate. Considerable research has primarily focussed on understanding the factors that contribute to maintaining the organism's survival within the production system (Evans and Sayers, 2000; Jacobs-Reitsma et al., 1995; Nauta and Havelaar, 2007; Rasschaert et al., 2007b; Russa et al., 2005). Whilst such work is important in gaining control of the organism, there appears to have been few studies that evaluate levels of knowledge about the organism held by those employed in the poultry production industry. Studies have typically focussed on working procedures rather than knowledge (e.g. identifying common risk factors surrounding *Campylobacter* spp. infections on farms; Bouwknegt et al., 2004). Similarly, a great deal of food safety knowledge research has been focused on consumers (Brennan et al., 2007; Haapala and Probart, 2004; Jevsnik et al., 2008a; McCarthey et al., 2007).

In part, this focus stems from the belief that consumers are the weakest link in an increasingly efficient food chain (Brennan et al., 2007). However, an employee's actions during the initial stages of the food chain may have considerable implications in reducing instances of food-borne diseases (Nel et al., 2004; Ansari-Lari et al., 2010). The industrial scale of much food production, particularly in broiler chickens, means that a comparatively small number of people are responsible for the safe supply of food to large sections of the population. This raises the prospect that unwitting actions of a small number of workers have the potential to cause serious threats to food safety practices as the improper working practices of food-handlers

may lead to the transfer of micro-organisms from the environment and from on or in the food-handler onto foodstuffs (Nel et al., 2004).

Knowledge gaps surrounding food safety practices, such as hand-washing, have been shown to have deleterious effects on food industry worker behaviour (Allwood et al., 2004). This paper examines the broiler industry employee knowledge levels of *Campylobacter* spp. through the use of questionnaires on a range of employees at one UK based integrator. The aim of this study was to elucidate the degree of knowledge employees held regarding sources of *Campylobacterosis* infection in humans and bacterial control methods in broiler production.

There are a wide variety of diseases that can be transmitted as a result of poor hygiene practices in the initial stages of the food chain. These can range from environmental bacterial species such as *Campylobacter* and *Salmonella*, to human diseases such as Hepatitis A (Ansari-Lari et al., 2010). The concept of the widespread transmission of pathogens to consumers via foodstuffs is particularly relevant for pathogenic bacteria.

This study focuses on *Campylobacter* spp., as currently the bacterium is the subject of major EU and UK government initiatives. The Food Standards Agency (FSA) has set a 50% reduction target in the prevalence of UK produced broiler meat by 2010 (FSA, 2008). Other studies have identified poultry processing as an area for preventative actions to reduce the risk of *Campylobacterosis* infection for consumers (Nesbakken and Skjerve, 1996).

Workers from other sectors of the food chain have been the subject of previous studies, such as kitchen staff in restaurants (Bolton et al., 2008; Ko, 2010) and food handlers employed in food processing (de Wit et al., 2007; Gomes-Neves et al., 2007; Jevsnik et al., 2008b). The principle finding of previous studies of food handlers in the meat food chain is that there is insufficient knowledge about certain aspects of food safety held by industry employees (Nel et al., 2004; Ansari-Lari et al., 2010). Even in studies that found adequate food safety knowledge levels, what an individual did in practice didn't always concur with this thinking (Ko, 2010). Indeed Raspor (2008) suggests that insufficient knowledge of food safety awareness in

production workers, coupled with similar knowledge gaps and poor practices amongst consumers, accentuates the risk to the latter.

This study was designed to produce baseline information to be used in company staff training programs. This is premised on the idea that employees who come into contact with broilers, and have little or no knowledge about the bacterium and its epidemiology, may ultimately hinder attempts to minimise the prevalence of the bacteria upon finished poultry products (Jevsnik et al., 2008b).

## 2.3 Materials and methods

### 2.3.1 Sampling technique – Questionnaire

A major UK broiler integrator was recruited to the study. Questionnaires (Appendix 1) were distributed to three distinct professional groups within the company: farmers, factory employees, and non-production workers. Farm workers included all of those who had daily contact with the broilers growing on farms (both farm workers and managers, Table 2.2). Factory workers were sampled from those who worked on or directly supervised the production line at a slaughterhouse which processed approximately 45,000 birds per day. Non-production workers were included as a control group and included the company's transport, senior management and office-based staff who do not have direct daily contact with the birds.

The questionnaire was self-administered and followed procedures followed in similar studies (Ansari-Lari et al., 2010; Gomes-Noves et al., 2007) with two key managers facilitating their distribution and collection. A draft questionnaire was piloted using university students (*n*=15), with improvements and amendments implemented thereafter as in other studies (Jevsnik et al., 2008a; McCarthey et al., 2007).

Prior to using the questionnaire on a multicultural group of respondents, formal, validated translations need to be obtained (Bullinger et al., 1998). A validated, formally translated version of the questionnaire was made available to respondents in English and Welsh, the latter being the first language of a number of factory employees and the majority of farmers in the study. This translated version was then checked both by the authors and by additional first-language Welsh speakers for

consistency and correct terminology, and thus should give consistent responses and therefore valid results, similar to other multi-lingual studies (Eertmans et al., 2006).

Respondents were assured of their anonymity being protected. This is important to ensure questions are answered truthfully. Respondents were identified only by job activity, age-group, gender and education, in the same way as a number of other studies (Gomes-Neves et al., 2007; Jevsnik et al., 2008a). The collection of questionnaires was facilitated by two integrator managers responsible for agricultural and factory aspects of the business. One question (results shown in Table 2.4) was adapted from an *E. coli* O157 food-safety questionnaire (R.E.L.U. *E. coli* O157 Project), and similar questions have been posed in other food-safety studies (Bolton et al., 2008).

### 2.3.2 Questionnaire Analysis

Questionnaire responses were analysed in a number of ways. Firstly the responses for each question were totalled to investigate the range and proportion of answers. Secondly a mark scheme was developed in order to attempt to assess the level of knowledge that respondents possessed.

#### 2.3.2.1 – Scoring System

Multiple choice questions were used with three to six possible answers, ranging from "none" to "a lot" and also including "don't know" in order to minimise the possibility of guessing the correct answer (Gomes-Neves et al., 2007). Furthermore, questions were structured so that simply by answering "a lot" to all sections, this would not give a high overall score.

#### 2.3.2.2 – Mark Scheme Data Sources

A mark scheme was produced for a number of the knowledge-based questions using findings and knowledge from peer-reviewed literature (Table 2.1). For each question the best answer was given 1 mark, with some questions carrying ½ a mark if applicable: for instance the difference between "a lot" and "quite a bit" can be subjective depending on an individual's interpretation.

Thus each questionnaire was marked to give a score out of 43 and added to the response data in the spreadsheet, in a similar manner to other studies (Brennan et al., 2007).

**Table 2.1** – Data sources used in compiling questionnaire mark scheme

| Question | References |
|---|---|
| 1 | Not Applicable |
| 2 | Not Applicable |
| 3 | Not Applicable |
| 4 | Not Applicable |
| 5 | Not Applicable |
| 6 | EFSA (2004) |
| 7 | EFSA (2004) |
| 8 | EFSA (2004); Kitchen and Vaughn (2007); MacCallum et al. (2005); Mellits et al. (2002) |
| 9 | Not Applicable |
| 10 | EFSA (2004); Mellits et al. (2002); Nauta and Havelaar (2007); Newell (2002) |
| 11 | Allen et al. (2008b), (2008c); Arsenault et al. (2007); Berndtson et al. (1996a); Berrang and Dickens (2000); EFSA (2004); Evans and Sayers (2000); Hald et al. (2007); Kazwala et al. (1992) |
| 12 | EFSA (2004); Hald et al. (2007); Jacobs-Reitsma et al. (1994); Refrégier-Petton et al. (2001); |
| 13 | EFSA (2004); Stern et al. (1995) |
| 14 | Berrang and Dickens (2000); EFSA (2004) |

### 2.3.3 Data Analysis

Data was analysed using SPSS 12.0 (SPSS Inc., Chicago, IL). A code was devised for the independent variable (e.g. job description), with the second column containing the questionnaire score. The dataset was checked for the homogeneity of

variance by calculating the Levene's Statistic. One-way ANOVAs ($p = 0.05$) were conducted for age group, education and job area, and an independent samples T-test for gender. Post-hoc analysis was conducted using Tukey tests for the one-way ANOVAs ($p = 0.05$).

## 2.4 Results and Discussion

### 2.4.1 Basic Responses

The overall questionnaire responses and some key features have been summarised in Tables 2.3 – 2.6. Firstly, 76.3% of respondents answered they knew "a little" or less about *Campylobacter* (Table 2.3), with 35% admitting they knew nothing, even the name, about the bacteria, (Table 2.3).

Approximately 90% of respondents correctly identified cross-contamination as being an important aspect of food safety. Opinions were divided regarding the harm of *Campylobacter* to humans with 30% of respondents admitting to not knowing anything about the potential harmful effects of *Camplobacter* infections. Whilst most respondents (74.2%) correctly identified two, or more of the possible symptoms of *Campylobacter* (out of a possible 6), no respondents correctly identified all six.

Only 5.4% of respondents correctly identified the peak period for *Campylobacter* spp. infections in poultry as being "late summer/autumn", although 52.7% indicated "summer". Whilst this is not crucial as workers should be aware of the food safety risks throughout the year, this further illustrates the lack of specific knowledge about the *Campylobacter* spp.

For questions about specific bacterial epidemiological knowledge, 46.2% correctly answered that the number of contaminated birds increased during transport, but only 35.5% for bacterial counts also increasing. This suggests the greater understanding of the bacteria's epidemiology needed for the latter, due to the complex effect of feed withdrawal upon gastro-intestinal (GI) tract contents and bacterial populations, rather than the simple visible fact that birds will contaminate each other with faeces when in transport crates.

When the same questions were posed regarding the processing line within the factory only 9.7% of respondents answered correctly indicating that the numbers of *Campylobacter* spp. decreased along the production line, indicating a lack of knowledge about how the bacteria reproduces (i.e. only in an animal's gastro-intestinal tract, and not outside in the environment). Forty percent indicated numbers increased, perhaps reflecting prior knowledge of other species of bacteria such as *Pseudomona* spp.

A similar question was also asked regarding how the number of contaminated birds changes along a production line. More than half of the respondents incorrectly answered that the number of birds increases (39.8%) or decreases (11.8%). These values are close to the results from the previous question on bacterial numbers, perhaps indicating an effect of the questionnaire layout, as they were two parts of the same question. Only 31.2% of respondents correctly identified that the number of contaminated birds stays roughly constant whilst travelling along the production line. All four of these questions had higher rates of unanswered scripts (16-20%), which is possibly an indication that respondents lost interest in answering as these questions were situated towards the end of the survey.

The responses to questions about how much individuals had heard about a range of bacteria are reported in Table 2.4. *Salmonella* spp. were the most recognised bacteria, with 61.3% of respondents indicating they had heard "quite a bit" or "a lot", followed by MRSA (55.9%), *Listeria* spp. (31.2%) with *Campylobacter* spp. fourth but some way behind at 24.7%. Similarly, *Campylobacter* spp. was fourth behind these bacteria, when the "nothing" responses were looked at, with a high level (53.8%) reporting that they failed to recognise *Campylobacter* spp. by name. The pseudo disease *Peruginella* (a concocted name by the authors) was identified as the least known disease (84.9%), which is reassuring as it was introduced to the list of diseases in order to act as a negative control.

This pattern is similar to other studies (Bolton et al., 2008), who when asking kitchen staff found 100% recognition of *Salmonella* spp., high levels for *Listeria* spp. and *Staphylococcus aureus,* but only 41.5% recognition of *Campylobacter* spp., despite it being a more prevalent food poisoning agent than the others in the UK (Evans and

Sayers, 2000). The study also found that head chefs who associated *Campylobacter* with poultry were significantly ($p < 0.001$) more likely to cook poultry meat correctly, indicating the importance of food safety education and how it can potentially impact food handlers' behaviour.

Responses about the sources of *Campylobacter* spp. infections are shown in Table 2.5. Whilst 40.8% and 47.4% of respondents correctly answered that poor hygiene and contact with animal faeces respectively were likely or very likely to be the source of an infection. Only 11.9% correctly identified raw vegetables and 15.1% pets as being a possible source. Of course, this is not directly applicable in broiler processing as neither are part of the production system, but never the less this shows a gap in food-safety knowledge. 53.7% and 37.6% identified undercooked meat and handling farm animals to be "likely" or "very likely" sources of infection, whereas studies have show that these sources are actually less likely. There was a high rate of unanswered sub-questions (26.2%) and respondents indicating "don't know" (20.1%).

The last area examined was the knowledge of those poultry production sectors considered important for pathogen control. Although the same questionnaire was used for all participants and the results collated (Table 2.6), it is more pertinent to focus on those areas which fall under the area of employment – i.e. production factors for farm employees and processing factors for factory employees. When assessing the farm employees' data the overall impression is quite favourable as high levels of correct responses were identified. For instance, there was the correct response of "a lot" from 91% and 75% for the areas of hand washing and footwear respectively. Only 25% of farm workers correctly identified the level of importance for both equipment and clothing (correct answers "quite a bit" and "quite a bit" or "some", respectively). However for both of these 58.3% and 66.7% responded "a lot", which whilst not correct under the mark scheme, does mean that the areas would not be overlooked as areas for pathogen control. Feed is a minimal area of concern for the control of the bacteria, but only 16.7% correctly identified this and 50% of respondents assessing it as "a lot", possibly drawing on their background knowledge on other pathogens such as *Salmonella* spp.

High numbers of responses were correct in identifying litter, house sanitation post-harvest and insect/rodent control to be important for the control of *Campylobacter* spp. (50%, 53.8% and 58.3% respectively). However, whilst 50% of respondents correctly identified the catching team as an important area of control of the pathogen, only 33% identified thinning, a process carried out by the catching team as being important for the control of *Campylobacter* spp.

Finally, employees appear to have limited knowledge regarding feed withdrawal as only 16.7% assessed this correctly as either "quite" or "somewhat" important. However, there is conflict between late feed withdrawal (for minimising *Campylobacter* spp. spread due to G.I. tract composition changes and increased risks of crop rupturing during processing; Hargis et al., 1995; Northcutt et al., 2003) and the requirement for the factory to have clean crops prior to slaughter.

For the 'control of the pathogen' questions relating to different processes within the factory, and hence particularly relevant to factory workers, correct answers were at low levels. Just 13.6% giving the correct answer for stunning and 6.8% for slaughter (both "none"), 4.5% for plucking, 4.5% for chilling 4.5% (both "quite a bit") and even evisceration was only 34.1% ("a lot").

However, if the marking scheme was made slightly more flexible a different pattern emerged. In order to analyse these responses to the three critical areas (plucking, evisceration, and chilling) were disaggregated into worker and manager categories, and the responses "quite a bit" combined with "a lot" to take into account minor response variations. Analysis of these showed an increased demonstration of knowledge, with 30.6%, 38.9% and 41.7% of workers identifying the importance of these areas for plucking, evisceration and chilling respectively. Factory managers faired even better, with their respective answers being 50%, 50% and 37.6%. The practice of hand-washing was correctly identified by 72% of factory workers, though only 37% of factory managers did likewise.

### 2.4.2 Results tables

**Table 2.2** – Summary of scores broken down by demographic questions (score out of 43 ± SEM)

| Employment | n= | Average Score |
|---|---|---|
| Farm Worker | 9 | 17.0 ± 1.6 |
| Farm Manager | 3 | 16.8 ± 1.7 |
| Factory Workers | 36 | 11.1 ± 0.9 |
| Factory Manager | 8 | 9.7 ± 2.6 |
| Office Staff | 32 | 13.6 ± 1.3 |
| Transport | 2 | 13.8 ± 1.3 |
| Senior Management | 3 | 16.0 ± 4.5 |

| Age Group | n= | Average Score |
|---|---|---|
| 16-24 | 25 | 8.4 ± 1.2 |
| 25-34 | 17 | 14.4 ± 1.4 |
| 35-44 | 18 | 13.6 ± 1.1 |
| 45-54 | 22 | 15.0 ± 1.2 |
| 55-64 | 11 | 15.0 ± 1.3 |
| 65+ | 0 | 0.0 ± 0.0 |

| Gender | n= | Average Score |
|---|---|---|
| Male | 51 | 12.7 ± 0.9 |
| Female | 37 | 13.1 ± 1.0 |
| Unanswered | 5 | 11.5 ± 2.2 |

| Education | n= | Average Score |
|---|---|---|
| Senior/High School | 41 | 13.3 ± 1.0 |
| College | 26 | 11.4 ± 1.2 |
| University | 19 | 13.6 ± 1.3 |
| Unanswered | 7 | 13.1 ± 2.8 |

**Table 2.3** – Summary of general responses to *Campylobacter* spp. questionnaire

**How much would you say you know about the bacteria *Campylobacter*? *n*, (%)**

| Nothing | Just the name | A little | Quite a bit | A lot | Expert | Unanswered |
|---|---|---|---|---|---|---|
| 33 (35.48) | 12 (12.9) | 26 (28.0) | 11 (11.8) | 7 (7.5) | 0 (0) | 4 (4.3) |

**How harmful do you believe *Campylobacter* is to humans? *n*, (%)**

| None | A little | Somewhat | Very | Deadly | Don't know | Unanswered |
|---|---|---|---|---|---|---|
| 5 (5.4) | 8 (8.6) | 10 (10.8) | 27 (29.0) | 11 (11.8) | 28 (30.1) | 4 (4.3) |

**How important is cross-contamination in food safety? *n*, (%)**

| Not at all | A little | some | Quite a bit | A lot | Don't know | Unanswered |
|---|---|---|---|---|---|---|
| 0 (0) | 1 (1.1) | 0 (0) | 8 (8.6) | 75 (80.6) | 3 (3.2) | 6 (6.5) |

**Identification of the human symptoms of *Campylobacter* infection (score out of 6) *n*, (%)**

| 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 10 (10.8) | 11 (11.8) | 17 (18.3) | 32 (34.4) | 20 (21.5) | 3 (3.2) | 0 (0) |

**In Europe, numbers of *Campylobacter* are at their peak in poultry during the following period?**

| Winter | Spring/E. Summer | Summer | Late summer/Autumn | Autumn | Unanswered |
|---|---|---|---|---|---|
| 9 (9.7) | 18 (19.4) | 46 (52.7) | 5 (5.4) | 0 (0) | 15 (16.1) |

**As chickens are transported to a slaughterhouse, do you think:**

| | Increase | Stay roughly constant | Decrease | Unanswered |
|---|---|---|---|---|
| *Campylobacter* Bacterial counts | 33 (35.5) | 38 (40.9) | 3 (3.2) | 19 (20.4) |
| Number of contaminated Birds | 43 (46.2) | 30 (32.3) | 3 (3.2) | 17 (18.3) |

**As chickens travel along the processing line, do you think:**

| | Increase | Stay roughly constant | Decrease | |
|---|---|---|---|---|
| *Campylobacter* Bacterial counts | 38 (40.9) | 28 (30.1) | 9 (9.7) | 18 (19.4) |
| Number of contaminated Birds | 37 (39.8) | 29 (31.2) | 11 (11.8) | 16 (17.2) |

**Table 2.4** – Summary of responses regarding recognition of bacteria

| **How much have you heard about the following bugs? *n* (%)** | | | | | | |
|---|---|---|---|---|---|---|
| Bacteria | nothing | A little | Quite a bit | A lot | Unanswered | *n* |
| *Salmonella* | 8 (8.6) | 28 (30.1) | 37 (39.8) | 20 (21.5) | 0 (0) | 93 |
| MRSA | 10 (10.8) | 29 (31.2) | 33 (35.5) | 19 (20.4) | 2 (2.2) | 93 |
| *Cryptosporidium* | 51 (54.8) | 24 (25.8) | 8 (8.6) | 5 (5.4) | 5 (5.4) | 93 |
| *B. subtilis* | 75 (80.6) | 10 (10.8) | 2 (2.2) | 1 (1.1) | 5 (5.4) | 93 |
| Norovirus | 60 (64.5) | 17 (18.3) | 10 (10.8) | 2 (2.2) | 4 (4.3) | 93 |
| *Perginella* | 79 (84.9) | 7 (7.5) | 1 (1.1) | 1 (1.1) | 5 (5.4) | 93 |
| *Clostridium* | 58 (62.4) | 18 (19.4) | 6 (6.5) | 3 (3.2) | 7 (7.5) | 93 |
| *Lactobacillis* | 66 (71.0) | 15 (16.1) | 3 (3.2) | 2 (2.2) | 7 (7.5) | 93 |
| *Listeria* | 34 (36.6) | 24 (25.8) | 22 (23.7) | 7 (7.5) | 6 (6.5) | 93 |
| *Shigella* | 70 (75.3) | 9 (9.7) | 3 (3.2) | 3 (3.2) | 8 (8.6) | 93 |
| *Campylobacter* | 50 (53.8) | 14 (15.1) | 11 (11.8) | 12 (12.9) | 6 (6.5) | 93 |

**Table 2.5** – Summary of responses regarding sources of *Campylobacterosis*

| **If you were to get food poisoning from Campylobacter, what are the most likely ways you would get it? *n*, (%)** | unlikely | a slight chance | likely | very likely | don't know | Unanswered |
|---|---|---|---|---|---|---|
| Drinking mains tap water | 28 (30.1) | 10 (10.8) | 5 (5.4) | 5 (5.4) | 20 (21.5) | 25 (26.9) |
| Handling farm animals | 5 (5.4) | 10 (10.8) | 24 (25.8) | 11 (11.8) | 19 (20.4) | 24 (25.8) |
| Contact with animal faeces (droppings) | 5 (5.4) | 5 (5.4) | 17 (18.3) | 26 (28.0) | 18 (19.4) | 22 (23.7) |
| Eating raw vegetables (e.g. salads) | 30 (32.3) | 11 (11.8) | 5 (5.4) | 6 (6.5) | 17 (18.3) | 24 (25.8) |
| Contact with other people | 20 (21.5) | 17 (18.3) | 6 (6.5) | 4 (4.3) | 19 (20.4) | 27 (29.0) |
| Streams, rivers ponds and lakes | 17 (18.3) | 15 (16.1) | 6 (6.5) | 3 (3.2) | 23 (24.7) | 29 (31.2) |
| Contact with household pets | 16 (17.2) | 17 (18.3) | 8 (8.6) | 6 (6.5) | 18 (19.4) | 28 (30.1) |
| Eating undercooked meat | 11 (11.8) | 3 (3.2) | 15 (16.1) | 35 (37.6) | 16 (17.2) | 13 (14.0) |
| From soil and mud | 19 (20.4) | 9 (9.7) | 8 (8.6) | 9 (9.7) | 20 (21.5) | 28 (30.1) |
| Air | 29 (31.2) | 12 (12.9) | 3 (3.2) | 3 (3.2) | 18 (19.4) | 28 (30.1) |
| Poor kitchen hygiene | 8 (8.6) | 9 (9.7) | 11 (11.8) | 27 (29.0) | 18 (19.4) | 20 (21.5) |

**Table 2.6** – Summary of knowledge regarding the control of *Campylobacter* spp. in broiler production

| How important do you think the following are for the control of pathogens in poultry production, specifically *Campylobacter* spp.? *n*, (%) | None | A little | some | Quite a bit | A lot | Don't know | Unanswered |
|---|---|---|---|---|---|---|---|
| Hand Washing | 2 (2.2) | 1 (1.1) | 0 (0) | 6 (6.5) | 67 (72.0) | 7 (7.5) | 10 (10.8) |
| Clothing | 3 (3.2) | 6 (6.5) | 3 (3.2) | 19 (20.4) | 38 (40.9) | 12 (12.9) | 12 (12.9) |
| Foot Wear | 3 (3.2) | 2 (2.2) | 7 (7.5) | 11 (11.8) | 45 (48.4) | 12 (12.9) | 13 (14.0) |
| Equipment | 3 (3.2) | 3 (3.2) | 3 (3.2) | 12 (12.9) | 45 (48.4) | 9 (9.7) | 18 (19.4) |
| Chicken Feed | 9 (9.7) | 4 (4.3) | 10 (10.8) | 9 (9.7) | 27 (29.0) | 19 (20.4) | 15 (16.1) |
| Chicken Drinking Water | 8 (8.6) | 9 (9.7) | 6 (6.5) | 13 (14.0) | 26 (28.0) | 17 (18.3) | 14 (15.1) |
| Chicken litter | 6 (6.5) | 4 (4.3) | 4 (4.3) | 13 (14.0) | 38 (40.9) | 13 (14.0) | 15 (16.1) |
| House sanitation after harvest | 7 (7.5) | 6 (6.5) | 3 (3.2) | 11 (11.8) | 40 (43.0) | 11 (11.8) | 15 (16.1) |
| Insect/Rodent Control | 3 (3.2) | 7 (7.5) | 5 (5.4) | 10 (10.8) | 39 (41.9) | 14 (15.1) | 15 (16.1) |
| Air in rearing shed | 10 (10.8) | 5 (5.4) | 7 (7.5) | 9 (9.7) | 24 (25.8) | 17 (18.3) | 21 (22.6) |
| Thinning | 8 (8.6) | 8 (8.6) | 4 (4.3) | 5 (5.4) | 21 (22.6) | 21 (22.6) | 26 (28.0) |
| Catching team | 8 (8.6) | 6 (6.5) | 6 (6.5) | 7 (7.5) | 26 (28.0) | 17 (18.3) | 23 (24.7) |
| Feed withdrawal | 12 (12.9) | 8 (8.6) | 4 (4.3) | 6 (6.5) | 19 (20.4) | 21 (22.6) | 23 (24.7) |
| Transport | 8 (8.6) | 8 (8.6) | 8 (8.6) | 9 (9.7) | 24 (25.8) | 17 (18.3) | 19 (20.4) |
| Stunning | 13 (14.0) | 9 (9.7) | 5 (5.4) | 4 (4.3) | 23 (24.7) | 19 (20.4) | 20 (21.5) |
| Slaughter | 10 (10.8) | 8 (8.6) | 8 (8.6) | 4 (4.3) | 25 (26.9 | 17 (18.3) | 21 (22.6) |
| Plucking | 9 (9.7) | 8 (8.6) | 7 (7.5) | 6 (6.5) | 26 (28.0) | 17 (18.3) | 20 (21.5) |
| Evisceration | 8 (8.6) | 2 (2.2) | 5 (5.4) | 13 (14.0) | 32 (34.4) | 17 (18.3) | 16 (17.2) |
| Chilling (air) | 14 (15.1) | 3 (3.2) | 3 (3.2) | 8 (8.6) | 28 (30.1) | 18 (19.4) | 19 (20.4) |

### 2.4.3 Analysis of Questionnaire Scores

Analysis of the scores for the different demographic sections (Table 2.2) showed a number of interesting features. After combining the job description groups into farm, factory and office as described previously, the Levene's statistic was found to be 0.127, i.e. above 0.05. Therefore we can assume the dataset has equal variances.

There was a significant difference in overall scores ($p$=0.003) between the groups (farm, factory and office workers). Post-hoc analysis indicated that difference was between farm worker scores and factory worker scores, but not between office

workers and either of the other categories. Whilst no statistical significance was found for the effects of gender ($p = 0.76$) or level of education ($p = 0.59$), for age group there was a difference ($p < 0.01$) between the youngest age-group (16-24) and all others, with post hoc tests confirming this ($p < 0.03$).

This indicates that farm employees have a better understanding of the issues surrounding *Campylobacter* spp. control than factory employees. This greater awareness of the issues could be due to farm employees having a better understanding of bio-security as a result of recent avian influenza outbreaks and/or other pathogen control programs such as *Salmonella* spp., rather than necessarily understanding the issues surrounding *Campylobacter* spp. This is supported by the high level (66.6%) of answers from farm employees saying they know nothing about *Campylobacter* spp.

The significant difference between the scores from the youngest age-group and the other groups indicates either a lack of knowledge about *Campylobacter* spp. or the absence of a serious approach to answering the questionnaire. Other studies have identified similar differences in the degree of knowledge gaps in food safety for similarly aged groups (14-19) and adults (Sanlier, 2009).

## 2.5 Conclusions

This study has demonstrated that broiler company employees possess some food-safety knowledge, with farm-based employees demonstrating significantly more knowledge about *Campylobacter* spp. than factory based employees. Overall knowledge levels were relatively low, (the highest mark achieved was only 53.5% by a farm worker and an office worker). Critical knowledge was at higher levels for items such as the importance of cross-contamination for food safety (90%), the importance of hand-washing (91%) and protective footwear (75%) to farm employees for the control of *Campylobacter* spp. (and/or other food-borne pathogens). This suggests that an important problem is currently confronting the industry as evidenced by the 25% of farm employees who were unaware of the importance of bio-secure footwear in the control of *Campylobacter*. Successful contamination of an entire broiler flock requires only a few bacteria. One employee

not following accepted hygiene procedures presents an important challenge to the efforts of the farm to maintain clean flocks (Berndtson et al., 1996a). When a quarter of the employees are unaware of procedures then contamination becomes highly probable.

Another knowledge gap identified in farm employees was the issue of thinning, which has the potential to increase infection risk from *Campylobacter* spp. . Whilst employees demonstrated that they were able to identify many other factors, a lack of focus on hygiene measures at thinning (resulting from a lack of understanding as to their importance for the control of *Campylobacter* spp.) can increase the risk of flock infection dramatically (Allen et al., 2008c).

Other studies assessing meat-processing workers have found similar results, with high levels of awareness of the critical role of general safety measures (97.9%), but far lower knowledge regarding microbial food hazards (67-78%) (Ansari-Lari et al., 2010). Studies have also found that basic hygiene practices such as wearing overalls, foot sanitation together with cleaning and disinfection procedures are understood and carried out routinely (Nel et al., 2004). Similarly, other studies of consumers found high levels of food safety knowledge and best practice knowledge (77.1% and 75.2% respectively), but lower levels of food science knowledge (43.7%), indicating that knowledge of what to do can be held without knowing the reasons why (McCarthey et al., 2007).

Interesting, when compared to a generic HACCP study regarding broiler chicken production and processing, there were few knowledge gaps that coincided with critical control points. The only incidence of this was the at the acceptance of live birds for slaughter stage, where knowledge gaps regarding farm workers (i.e. the importance of clothing, footwear and equipment hygiene both in workers and in catchers) could increase the likelihood of *Campylobacter* spp. contaminated birds entering a processing factory.

Interestingly *Campylobacter* spp. were not mentioned in the study at any point (unlike *Salmonella* spp.). This could therefore indicate that some HACCP studies used within the broiler industry could need reviewing in order to incorporate

procedures for the control of *Campylobacter* spp. One notable inclusion would be the addition of evisceration as critical control point, as poor control of this process, leading to increased viscera rupture, would increase faecal content spread across carcasses and result in increased numbers of *Campylobacter* spp. upon a broiler carcass (Berrang et al., 2004).

Another point of note was that a number of the process steps, such as refrigerating carcasses to between 4 and 10°C, together with decreasing the oxygen content within sealed packs of poultry products to less than 5%, would actually favour the survivability of *Campylobacter* spp. The bacterium is susceptible to the level of oxygen typically found in air and indeed favours levels between 3 and 15% (Krieg and Hoffman, 1986). It also survives for longer periods of time in cold water than warmer water (>15°C) (Skirrow, 1991), which is particularly pertinent due to the large volumes of water that are used during poultry processing.

### 2.5.1 Further Work

In addition to reviewing existing HACCP studies, this work could easily be taken further in a number of different areas. Firstly the results of this study could be used as a baseline for food hygiene seminars/workshops held within the company. Training from company experts and supervisors has been reported to be the most effective (Jevsnik et al., 2008b), and thus a co-ordinated scheme for managers and supervisors could be implemented, with the aim that they would disseminate this in practice to those who they had responsibility for.

Plausibly this would be separated farm and factory workers, focusing on their respective areas of employment in order that attendees were not overloaded with information, and instead concentrated on the knowledge gaps identified, with basic explanations of the science behind it. However, broiler-integrator senior managers could determine whether this would be a *Campylobacter* spp. focused exercise or would fit within the wider framework of food-safety, depending on their perceived requirements.

Other studies have found that the primary focus of food-safety education programs should be the basics such as hand-washing and avoiding cross-contamination, with

secondary messages being more complicated and scientific (Medeicos et al., 2001). Additionally, studies have shown perceived vulnerability to foodborne disease to have an effect upon an individual's behaviour (Redmond and Griffith, 2004). The same study found the recognition of personal control and responsibility for food-safety to be a prerequisite for an individual to initiate the appropriate food-safety behaviours. Therefore, illustrating the link between an individual's actions and the disease consequences within the consumer could prove to be a powerful method of reinforcing the general food-safety message.

Additionally, a survey of actual practices could be conducted by a combination of interviews, questionnaires and/or observations. The aim of this would be to determine whether actual practices within the food chain followed what employees said and thought best-practice was. Others (Daniels et al., 1998) found that 99% of observed consumer participants made at least one major hygiene error whilst preparing food, which would have serious ramifications if found to be a similar situation within an industrial setting. Other work has shown a negative correlation ($p = 0.05$) between food-safety knowledge and practice (Ansari-Lari et al., 2010), indicating that increased knowledge alone does not always give the expected results in food handling behaviours.

Employee attitude and job satisfaction could therefore be a secondary avenue of investigation. A number of studies have linked positive attitudes and satisfaction with best practice, even so far as using job satisfaction to predict an individual's food safety perception and practice (Ko, 2010). Other benefits of having a workforce with a positive attitude have also been observed, such as increased welfare positive behaviours (Coleman et al., 2003), productivity and employee loyalty (Ko, 2010), fostered by creating a positive motivational atmosphere within the workplace.
The high level of non-responses to certain questions (15.4% overall, but as high as 31.8% for the critical control areas for factory employees) could indicate that attitudes towards food safety are poor within certain individuals, which should certainly be something investigated more thoroughly by the company's management. As mentioned previously, only a few instances from a few individuals are needed to create a serious food-safety risk.

Finally, it is worth noting that these educational programs and further surveys are only useful for the specific control of *Campylobacter* spp. in conjunction with other programs, such as water/feed additive research, Unless the prevalence of *Campylobacter* spp. in UK flocks decreases dramatically, then a stand-alone education program is likely to have little if no effect, but in combination with these, education could prove to be one of the foundation stones for the effective control of the bacterium.

## 2.6 Acknowledgements

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# Chapter 3

## Lime-treatment as an effective sanitization method for *Salmonella* spp. and *E. coli* O157:H7 in abattoir blood waste

## 3.1 Abstract

Historically, abattoir blood waste was disposed of via land-application. However, the EU Animal By-Products Regulations (1774/2002) necessitates it to be rendered or incinerated, resulting in significant producer costs and the loss of a valuable soil amendment. However, land-spreading of pre-treated waste may be approved if it has been shown that the treatment eradicates pathogens and that land application of the resulting waste is of agricultural benefit. Fresh poultry blood waste was treated with varying doses of lime ($CaO$ or $Ca(OH)_2$) and total viable counts (TVC) of bacteria and the survival of *Salmonella poona* and *Escherichia coli* O157 monitored over time. Lime application increased blood pH to > 12.0 and was found to be highly effective in eradicating pathogens ( > 8 $\log_{10}$ reduction) and reducing TVC numbers, even at low rates of lime treatment (2.5% w/v). Furthermore, the application of lime treated blood even at a low rate of 2.5 t $ha^{-1}$ significantly increased grass yield ($P < 0.001$). The findings of this trial provide strong evidence that lime treatment of abattoir blood waste followed by land-application is an effective method that should be considered for inclusion in the EU Animal By-Products Regulations (1774/2002).

## 3.2 Introduction

The introduction of the European Union (EU) Animal By-Products Regulations (1774/2002) outlawed land-spreading as a method of disposing untreated blood waste from abattoirs; stating that such waste must be rendered or incinerated (Anon, 2005). This has significant financial implications for meat processors in terms of costs for waste haulage and disposal; especially given the high intensity heat treatment that is required for incineration. Furthermore, this practice denies farmers from applying a proven fertilizer to their land (Abbas and Choudhary, 1995; Petroczki, 2004). Outside of the EU regulated area, treatments available for abattoir blood wastes include composting, management *via* wastewater treatment plants, and land-application. However, all such methods have a range of disadvantages either in terms of space and time requirements, costs, or risk of pollution (Avery et al., 2009; Bicudo and Goyal, 2003; Mittal, 2006; Stentiford, 1996).

Abattoir wastes can be potential pathways for human pathogens such as *Escherichia coli* O157 through contamination of agricultural land (Jones, 1999). Such pathogens may then survive and even thrive in the soil (Avery et al., 2005; Hepburn et al., 2002; Williams et al., 2007). If abattoir waste is subject to land-spreading, it should therefore be effectively pre-treated to eradicate pathogens. The optimum pH range for *E. coli* and *Salmonella* survival is between 6 and 7 (Mittal, 2006) and it has been shown that raising the pH to 12 for just 15 seconds can give a 8 $\log_{10}$ reduction in *E. coli* and *S. enteritidis* (Mendonca et al., 1994). Indeed, raising the pH of wastes *via* lime treatment is extensively used as an effective technique for killing pathogens in sewage sludge wastewaters (Bean et al., 2007; Boost and Poon, 1998). It has also been proven to effectively treat animal wastes (Duffy, 2003; Maguire et al., 2006; Ruiz et al., 2008) and recent work demonstrated how lime application eradicated *E. coli* O157 from abattoir waste (Avery et al., 2009).

Many farmers, particularly those with acidic soils, routinely add lime to their land in order to raise soil pH to levels which improves fertility by increasing the soil's nutrient availability, microbial activity and cation exchange capacity (Brady and Weil, 1999). Lime is typically applied as ground limestone, however processed lime in the form of CaO or $Ca(OH)_2$ can be used, particularly when rapid changes of pH are required (Brady and Weil, 1999). Land application of lime-treated blood waste

may therefore both effectively reduce pathogen numbers and provide valuable agricultural benefits in terms of a nutrient and liming resource.

Current guidelines from the UK Environment Agency state that wastes may be approved for spreading onto agricultural land if they demonstrate agricultural benefit and that the activity does not cause pollution or harm to health (Anon, 2008). Whilst limited work has demonstrated the ability of lime treatment to eradicate some pathogens from abattoir wastes (Avery et al., 2009), further work is needed to determine the effective dose rate and the agronomic benefits of subsequent land-application. The aims of this study were: 1) to determine whether adding lime to blood waste would effectively kill *E. coli* O157 and *S. poona*, and 2) whether the addition of the lime-treated blood waste would give an agricultural benefit when added to established grassland.

## 3.3 Materials and methods

### 3.3.1. Bacterial strain

An inoculum of two environmental isolates of *E. coli* O157:H7, #3704 (Campbell et al., 2001), #1290 together with a *lux*-marked strain #Tn*5 luxCDABE* (Ritchie et al., 2003), in stationary growth phase was prepared from three fresh overnight cultures (LB broth; Oxoid Ltd., Basingstoke, UK; 18 h, 37°C, 150 rev $min^{-1}$). Whilst none of these strains actively produce toxins (Campbell et al., 2001), such strains are still representative of toxigenic strains survival patterns (Kudva et al., 1998; Ritchie et al., 2003). To form the inoculum, cells were washed and concentrated as described in Avery et al. (2005), then combined together in equal volumes. Numbers of cells were determined following plating of serial dilutions onto SMAC agar (Oxoid, UK) and counting colonies of characteristic *E. coli* O157 appearance following incubation (24 h, 37°C).

Similarly, a fresh culture of *S. poona* inoculum (#C6009) was prepared from an Oxoid Cultiloop stock using Buffered Peptone Water (Oxoid, UK) as the growth media, according to the manufacturer's instructions. Serial dilutions were subsequently plated onto xylose-lysine-desoxycholate agar (XLD; Oxoid, UK) and the numbers of *S. poona* ascertained following incubation (24 h, 37°C).

### 3.3.2 Blood waste samples

Poultry blood waste was collected from a large commercial poultry abattoir located in North Wales. This was collected from pooled blood on the floor of the slaughter area, and comprised of approximately 20% wash water, which had the additional benefit of preventing the blood from coagulating before testing. Such waste is classed as 'Category 3' material under the EU Animal By-Products Regulations (1774/2002) (Anon, 2005). Samples were transported to the laboratory and analysed within 30 min of collection. All subsequent analysis was done in triplicate, unless otherwise stated. Prior to the addition of the bacterial inoculums, the blood waste was tested for pH and electrical conductivity (EC) to monitor consistency.

### 3.3.3 Resistance of pathogenic bacteria to lime treatment

#### 3.3.3.1 Selection of levels of lime addition

Three treatments were investigated, namely calcium oxide ($CaO$; also known as Quicklime), calcium hydroxide ($Ca(OH)_2$; also known as Slaked lime or Hydrated lime) and a calcium control, calcium chloride ($CaCl_2$).

Preliminary trials of 0.1%, 1% and 10% (w/v) of CaO added to blood waste showed that whilst levels of 10% were effective in inhibiting *Salmonella* spp. 0.1% was not. However at 1% CaO *Salmonella* spp. were periodically isolated (0, 20, 40, and 100 min – Table 3.4) meaning that the effective level for controlling pathogenic bacteria such as *Salmonella* spp. lay between 1% and 10%. At this upper level of 10% the blood-lime mixture solidified, which would render the end product difficult to handle and dispose in a commercial situation. Thus it was decided to investigate the effectiveness of three addition rates for all treatments, namely 2.5%, 5% and 7.5% (w/v), together with 0% addition.

Prior to commencing the trial it was envisaged that $CaCO_3$ would be included as one of the treatments. However, in the preliminary trials $CaCO_3$ was found to be ineffective as a chemical treatment for blood waste as it only partially dissolves in the blood waste. Additionally, it failed to raise the pH above 9.0 and gave no inhibitory properties against the bacteria (data not shown). Therefore it was decided not to include it in the full-scale trial.

3.3.3.2 Addition of lime

Blood waste was mixed with each lime treatment in triplicate 50 $cm^3$ polypropylene vessels. This mixture was then homogenised thoroughly by hand (15 s), before adding 1 ml of *E. coli* O157:H7 inoculum and 1 ml of *S. poona* inoculum. After this, luminescence was immediately determined and then at 15 min intervals for 2 h using a Model 18172 luminometer (Nova Biomedical, Waltham, MA, USA), with the treatments being placed on an orbital shaker (120 rev $min^{-1}$, 37°C) between sampling. Simultaneously, solution pH was also determined over the 2 h experimental period.

3.3.3.3 Bacterial enumeration

Immediately after lime and pathogen addition, 100 µl of the mixture was removed and serially diluted in quarter-strength Ringer solution (Oxoid). Relevant dilutions were then plated onto Standard Plate Count Agar (Oxoid) and incubated at 37°C for 24 h prior to enumeration of characteristic colonies to determine total viable bacterial counts (TVC). An additional 500 µl of the lime, blood and pathogen mixture was added to 4500 µl of both Tryptone Soya Broth (TSB; Oxoid) and Buffered Peptone Water (BPW; Oxoid) and placed on an orbital shaker (120 rev $min^{-1}$, 37°C, 24 h) to enrich bacterial numbers prior to analysis for the presence/absence of *E. coli* O157 and *Salmonella* spp., respectively. Both of these samples were repeated every 20 min for the duration of the experiment, once again being placed on an orbital shaker (120 rev $min^{-1}$, 37°C) in-between sampling.

Subsequently, 100 µl of the TSB sample was plated onto SMAC agar and further incubated as described previously before examining for typical *E. coli* O157 colonies; which were confirmed via latex agglutination (Oxoid DR 0620). Sampling was repeated every 20 min for the duration of the experiment, and kept in an orbital shaker (120 rev $min^{-1}$, 37°C) between samples being taken.

Following incubation, the enriched BPW sample was pipetted into 10 ml of Rappaport-Vassiliadis Medium (RV; Oxoid) and 10 ml of Muller Kaufmann tetrathionate novobiocin broth (MKTTn; Oxoid) at a rate of 100 µl of sample for RV and 1000 µl for MKTTn. These were then further incubated for 24 h at 41.5°C and

37°C, respectively. One 10 μl loopful of both of these selective media were sub-cultured onto XLD agar (Oxoid) and Brilliant Green Agar (BGA, Oxoid). These were then further incubated for 24 h at 37°C. On both agars, presumptive colonies were confirmed via a latex agglutination test kit (Oxoid FT 0203).

### 3.3.4 Effect of lime treated blood on grass growth

The effect of lime treated blood was investigated by adding a consistent level of CaO (10g $l^{-1}$) to varying levels of blood waste (equivalent to 0, 2.5, 12.5 and 25 t $ha^{-1}$). Deionised water was used to make all treatments up to the same volume (equivalent to 25 t $ha^{-1}$). Additionally a negative control was used containing this equivalent quantity of water with no CaO.

Field-moist soil (Eutric cambisol) was placed in pots (6.5 cm diameter) at a bulk density of 1 g $cm^{-3}$, sown with perennial ryegrass (*Lolium perenne* L.) at a typical agricultural sowing rate of 35 kg $ha^{-1}$ and left to establish for four weeks. Further physicochemical and biological details of the soil can be found in Roberts et al. (2006). Subsequently, each treatment was injected 5 cm below the soil surface at rates to mimic commercial practices (Chadwick and Laws, 2002). The trial was conducted in a greenhouse with automatic watering and a 16 h photoperiod.

### 3.3.5 Data analysis

All statistics for the experiment were processed using SPSS 12.0.1 (SPSS Inc., Chicago, IL). Homoscedasticity was tested for using Levene's test, and when confirmed, one and two-way ANOVAs were used (plant biomass and pH respectively). Where heteroscedasticity was found (TVC data and shoot weight), the use of ANOVAs has been reported to be unreliable (Ananda and Weerahandi, 1997). Therefore a Kruskal-Wallis was used for the shoot weight data, and a Friedman's test for the TVC data in place of a Two-way ANOVA (Theodorsson-Norheim, 1987). Post-Hoc analysis was conducted using Tukey tests for homoscedastic data and Dunnett's C test for heteroscedastic data.

## 3.4 Results

### 3.4.1 Waste characteristics

Due to the intensive nature of the laboratory work, each of the lime treatment replicates was tested on a separate day. Thus the blood waste characteristics were monitored for consistency (Table 3.1). Initial pH values were all around pH 7 (mean 6.9 ± 0.32 s.d.), though EC was more variable. Whilst most EC values were in the range of 15 to 25 mS $cm^{-1}$, each of the three treatments had outliers. Although these were higher than the other values, it would seem that they indicate slightly lower water contents, and reflect that in practice these slight variations would occur from day to day at abattoirs.

**Table 3.1** – Summary of blood pH and electrical conductivity prior to treatment with lime.

| Chemical | Replicate | Initial pH | Electrical conductivity (mS $cm^{-1}$) |
|---|---|---|---|
| CaO | 1 | n/a | 24.6 ± 0.36 $^{a}$ |
| | 2 | 7.09 | 99.9 ± 1.35 $^{b}$ |
| | 3 | 6.92 | n/a |
| $Ca(OH)_2$ | 1 | 7.38 | 79.0 ± 4.01 $^{c}$ |
| | 2 | 6.24 | 19.3 ± 0.62 $^{a}$ |
| | 3 | 6.97 | 15.4 ± 1.64 $^{a}$ |
| $CaCl_2$ | 1 | 6.84 | 88.2 ± 2.84 $^{c}$ |
| | 2 | 6.96 | 18.4 ± 0.19 $^{a}$ |
| | 3 | 6.80 | 22.4 ± 0.75 $^{a}$ |

Each conductivity value is the mean of three readings ± SEM
Superscript letters denote significant differences at the $P<0.05$ level
n/a – not available

### 3.4.2 Waste pH

A slight increase of up to 0.4 pH units was seen within the 0% control blood waste treatments over the 2 h trial period. All the different dose rates of CaO and $Ca(OH)_2$ raised the pH of the blood waste from around pH 7 to pH 12 or above (Table 3.2). This increase was initially very rapid (in the first few seconds after addition) but soon stabilised with little further increase observed over the course of the

experiment. In contrast, the $CaCl_2$ treatment caused an initial drop in waste pH in a dose dependent manner. After this initial decline in pH, levels slowly rose back to that of the control treatment.

**Table 3.2** – Summary of pH values over the course of the experiments.

| Calcium Treatment Type | % | Time (min) 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|
| CaO | 0 | 7.0 ± 0.1 | 7.3 ± 0.2 | 7.3 ± 0.2 | 7.4 ± 0.2 | 7.3 ± 0.2 | 7.3 ± 0.2 | 7.3 ± 0.2 | 7.3 ± 0.2 | 7.2 ± 0.2 |
| | 2.5 | 11.9 ± 0.0 | 12.5 ± 0.1 | 12.6 ± 0.1 | 12.6 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 |
| | 5 | 12.0 ± 0.2 | 12.8 ± 0.2 | 12.9 ± 0.2 | 13.0 ± 0.2 | 13.0 ± 0.2 | 13.0 ± 0.3 | 13.0 ± 0.2 | 13.0 ± 0.2 | 13.0 ± 0.2 |
| | 7.5 | 11.6 ± 0.1 | 12.2 ± 0.2 | 12.3 ± 0.2 | 12.4 ± 0.2 | 12.4 ± 0.2 | 12.5 ± 0.2 | 12.5 ± 0.2 | 12.5 ± 0.2 | 12.5 ± 0.2 |
| CaOH | 0 | 6.9 ± 0.0 | 7.2 ± 0.1 | 7.2 ± 0.1 | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.2 ± 0.1 | 7.1 ± 0.2 | 7.1 ± 0.1 |
| | 2.5 | 12.0 ± 0.1 | 12.4 ± 0.1 | 12.5 ± 0.1 | 12.6 ± 0.1 | 12.6 ± 0.1 | 12.6 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 | 12.7 ± 0.1 |
| | 5 | 12.0 ± 0.1 | 12.6 ± 0.3 | 12.7 ± 0.3 | 12.8 ± 0.3 | 12.9 ± 0.3 | 12.9 ± 0.3 | 12.9 ± 0.3 | 12.9 ± 0.4 | 13.0 ± 0.4 |
| | 7.5 | 12.1 ± 0.2 | 12.5 ± 0.3 | 12.6 ± 0.3 | 12.7 ± 0.3 | 12.8 ± 0.3 | 12.8 ± 0.3 | 12.8 ± 0.3 | 12.8 ± 0.3 | 12.9 ± 0.3 |
| CaCl | 0 | 6.9 ± 0.3 | 7.1 ± 0.3 | 7.1 ± 0.3 | 7.2 ± 0.3 | 7.1 ± 0.3 | 7.1 ± 0.3 | 7.1 ± 0.3 | 7.1 ± 0.3 | 7.1 ± 0.3 |
| | 2.5 | 6.1 ± 0.2 | 6.4 ± 0.2 | 6.5 ± 0.3 | 6.5 ± 0.3 | 6.5 ± 0.3 | 6.6 ± 0.3 | 6.6 ± 0.3 | 6.6 ± 0.3 | 6.6 ± 0.3 |
| | 5 | 6.0 ± 0.2 | 6.3 ± 0.2 | 6.3 ± 0.2 | 6.4 ± 0.2 | 6.4 ± 0.2 | 6.4 ± 0.2 | 6.4 ± 0.2 | 6.5 ± 0.2 | 6.5 ± 0.1 |
| | 7.5 | 5.7 ± 0.1 | 6.1 ± 0.1 | 6.2 ± 0.1 | 6.2 ± 0.1 | 6.2 ± 0.2 | 6.2 ± 0.1 | 6.2 ± 0.1 | 6.2 ± 0.1 | 6.2 ± 0.1 |

Each value shown is the mean of three replicates carried out in triplicate ± SEM.

Statistical analysis of the waste pH results (using a 2 way ANOVA followed by Tukey pair-wise comparison) showed a significant difference in pH change between the $CaCl_2$ and the CaO and $Ca(OH)_2$ treatments ($P < 0.001$), but not between the CaO and $Ca(OH)_2$ treatments ($P = 0.907$). Overall, increasing the contact time of the

different chemicals with the blood waste resulted in no significant change in pH after the initial measurement. Significant differences were also found between the treatment concentrations ($P < 0.001$), importantly between the 0% level and all others.

### 3.4.3 Microbiological validation of lime treatment

#### 3.4.3.1 Total viable counts (TVC)

There was greater than a 1 $\log_{10}$ decrease in TVC in the blood wastes treated with either CaO or $Ca(OH_2)$, whereas $CaCl_2$ had little overall effect on TVC (Table 3.3, Figure 3.1). The dataset was statistically analysed using a Friedman's Test due to unequal variances between groups. This showed a significant difference ($P < 0.001$) between the groups, with the CaO mean being the lowest of the mean ranks (1.33), followed by $Ca(OH_2)$ (1.83) and $CaCl_2$ being the highest (2.83). Post hoc tests showed there to be statistical differences between CaCl and both lime treatments and between the 0% level and all others ($P < 0.001$).

**Table 3.3** – Summary of Total Viable Count (TVC) testing of the blood/lime treatment

| Chemical | % | Time (min) | | |
|---|---|---|---|---|
| | | 5 | 65 | 125 |
| CaO | 0 | 8.03 ± 0.03 | 7.32 ± 0.13 | 7.16 ± 0.26 |
| | 2.5 | 6.20 ± 0.07 | 5.71 ± 0.04 | 6.11 ± 0.03 |
| | 5 | 6.00 ± 0.10 | 6.41 ± 0.04 | 5.68 ± 0.08 |
| | 7.5 | 6.13 ± 0.03 | 5.88 ± 0.08 | 5.98 ± 0.05 |
| $CaCl_2$ | 0 | 7.87 ± 0.07 | 7.74 ± 0.27 | 7.73 ± 0.36 |
| | 2.5 | 7.69 ± 0.04 | 7.78 ± 0.06 | 7.70 ± 0.14 |
| | 5 | 7.77 ± 0.08 | 7.70 ± 0.04 | 7.46 ± 0.12 |
| | 7.5 | 7.62 ± 0.11 | 7.76 ± 0.03 | 7.28 ± 0.13 |
| $Ca(OH)_2$ | 0 | 7.82 ± 0.05 | 7.63 ± 0.25 | 7.78 ± 0.29 |
| | 2.5 | 6.10 ± 0.01 | 6.28 ± 0.19 | 6.45 ± 0.22 |
| | 5 | 6.37 ± 0.04 | 6.34 ± 0.08 | 5.74 ± 0.16 |
| | 7.5 | 6.20 ± 0.04 | 6.20 ± 0.09 | 5.78 ± 0.29 |

Each result shown is the mean of three replicates ($\log_{10}$ CFU $ml^{-1}$ ± S.E.).

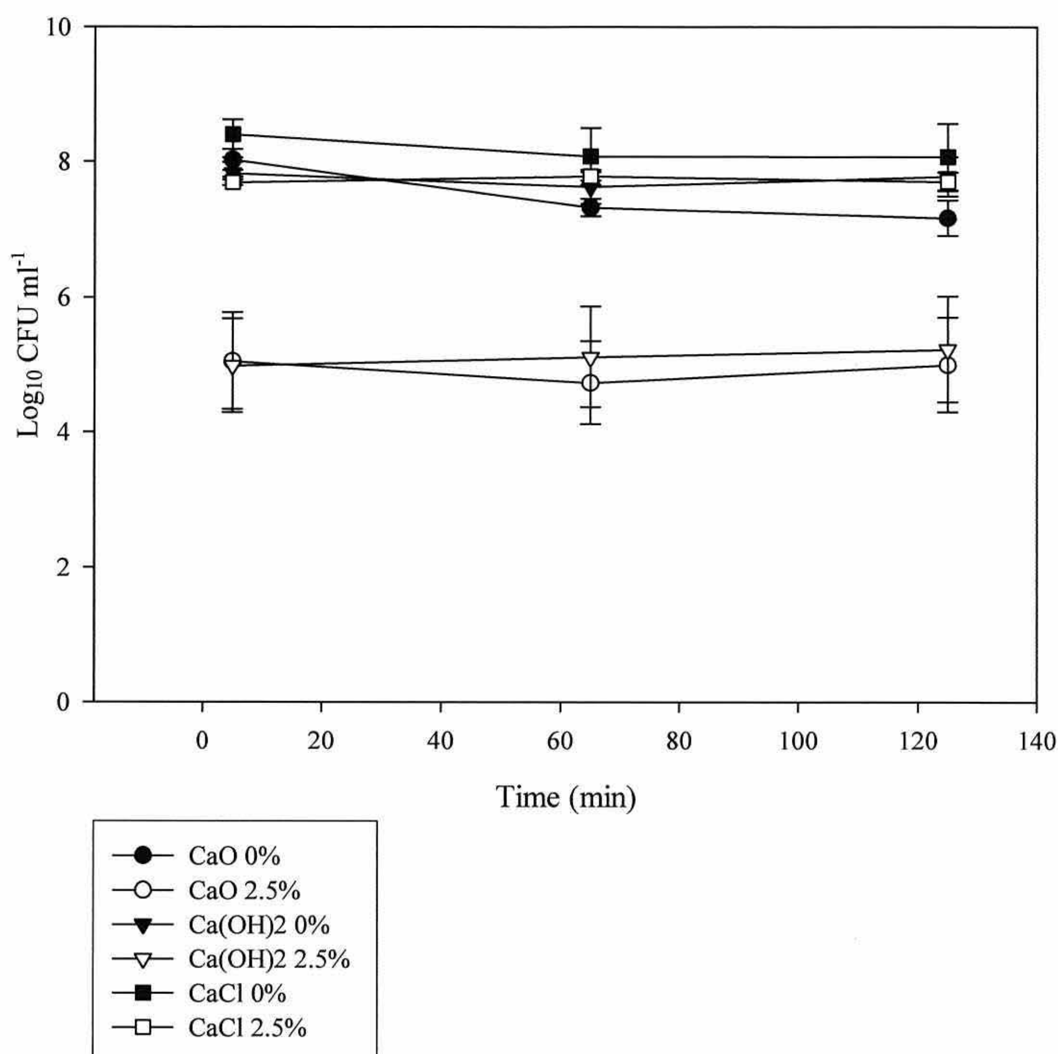


**Figure 3.1** - Total Viable Counts from calcium compound treated blood, sampled at the beginning, the middle and the end of the trials. Values represent the mean ± SEM of three replicate trials, with duplicate sampling for each trial ($n = 6$).

3.4.3.2 *Salmonella*

The *S. poona* inoculum added to the blood waste gave an initial population density of $\log_{10}$ 9.25 ± 0.10 CFU $ml^{-1}$. Throughout the 2 h experimental incubation period *S. poona* was successfully isolated from the untreated blood waste (Table 3.4). In contrast, analysis of the treated blood wastes showed that both CaO and $Ca(OH)_2$ were highly effective in killing *S. poona* bacteria. No viable cells could be recovered immediately after CaO and $Ca(OH)_2$ addition. This lack of detection was apparent even after enrichment. The control treatment and $CaCl_2$ treatment were ineffective in

killing the bacteria, however, as positive results were found throughout the duration of the trial.

**Table 3.4** – Summary of presence/absence of *Salmonella poona* and *E. coli* O157 in inoculated abattoir blood waste following lime-application.

| Chemical | % | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 20 | 40 | 60 | 80 | 100 | 120 |
| CaO | 0 | + | + | + | + | + | + | + |
| | 0.1 | + | + | + | + | + | + | + |
| | 1.0 | + | + | + | - | - | + | - |
| | 2.5 | - | - | - | - | - | - | - |
| | 5 | - | - | - | - | - | - | - |
| | 7.5 | - | - | - | - | - | - | - |
| | 10 | - | - | - | - | - | - | - |
| $Ca(OH)_2$ | 0 | + | + | + | + | + | + | + |
| | 2.5 | -* | -* | -* | - | - | - | - |
| | 5 | - | - | - | - | - | - | - |
| | 7.5 | - | - | - | - | - | - | - |
| $CaCl_2$ | 0 | + | + | + | + | + | + | + |
| | 2.5 | + | + | + | + | + | + | + |
| | 5 | + | + | + | + | + | + | + |
| | 7.5 | + | + | + | + | + | + | + |

Each result has been confirmed in triplicate for each species, and summarised – *S. Poona* was not found without the presence of *E. coli* O157, apart from CaO at 0.1, 1.0 and 10% where *E. coli* O157 was not tested for.

+ presence of *S. Poona* & *E. coli* O157

- absence of *S. Poona* & *E. coli* O157

* *E. coli* O157 detected in one replicate

3.4.3.3 *E. coli* O157

After inoculation, the blood waste was tested and found to contain $\log_{10}$ 9.41 ± 0.11 CFU *E. coli* O157 $ml^{-1}$. As occurred for *S. poona*, no viable *E. coli* O157 were recovered from any of the blood wastes to which CaO or $Ca(OH)_2$ had been added, even after enrichment (Table 3.4), apart from one $Ca(OH)_2$ replicate during the initial three sampling time points, though not subsequently. Again, $CaCl_2$ was not effective in reducing the population density of the pathogen.

3.4.3.4 Luminescence

Whilst the *lux*-marked strain of *E. coli* O157 showed high levels of activity within the unamended treatment (control 0% level) and the $CaCl_2$ control, the addition of CaO and $Ca(OH)_2$ quickly reduced this activity to below detectable levels (Table 3.5). Although there was an initial reduction in luminescence from the $CaCl_2$ treatment, this was relatively small and subsequently the values increased over the remaining duration of the experiment (Table 3.5).

**Table 3.5** – Summary of bioluminescence values (in R.L.U.s) from the *lux*-marked *E. coli* O157 strain.

| Calcium Treatment Type | % | Time (min) 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|---|---|---|---|---|---|---|---|---|---|---|
| CaO | 0 | 1379 ± 563 | 1015 ± 454 | 939 ± 420 | 971 ± 434 | 890 ± 398 | 861 ± 385 | 824 ± 369 | 782 ± 350 | 738 ± 330 |
| | 2.5 | 67 ± 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 35 ± 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7.5 | 22 ± 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CaOH | 0 | 2948 ± 1114 | 2695 ± 1347 | 2460 ± 1230 | 2239 ± 1120 | 1803 ± 901 | 2349 ± 888 | 2386 ± 902 | 2328 ± 880 | 2165 ± 818 |
| | 2.5 | 17 ± 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 23 ± 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7.5 | 9 ± 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CaCl | 0 | 1627 ± 615 | 1916 ± 724 | 1918 ± 725 | 1745 ± 660 | 1753 ± 663 | 1591 ± 601 | 1564 ± 591 | 1569 ± 593 | 1508 ± 570 |
| | 2.5 | 1155 ± 437 | 1727 ± 653 | 1758 ± 665 | 1864 ± 705 | 1821 ± 688 | 1645 ± 622 | 1787 ± 676 | 1625 ± 614 | 1572 ± 594 |
| | 5 | 362 ± 137 | 720 ± 272 | 871 ± 329 | 969 ± 366 | 1022 ± 386 | 1112 ± 420 | 1264 ± 478 | 1241 ± 469 | 1219 ± 461 |
| | 7.5 | 367 ± 139 | 483 ± 183 | 577 ± 218 | 680 ± 257 | 718 ± 271 | 824 ± 312 | 806 ± 304 | 806 ± 305 | 896 ± 339 |

Each result shown is the mean of three replicates, carried out in triplicate ± SEM.

### 3.4.4 Effect of lime treated blood on grass growth

Analysing the shoot and total Biomass data sets (using Kruskal-Wallis and 1-way ANOVA, respectively) showed significant differences for both DM weights ($P < 0.01$).

Post-Hoc (Dunnett C) tests on shoot DM mean values revealed significant differences ($P < 0.05$) between the negative control and both 12.5 t $ha^{-1}$ and 2.5 t $ha^{-1}$ treatments. Similar significant differences were found between the 0 t $ha^{-1}$ treatment and the 12.5 t $ha^{-1}$ and 2.5 t $ha^{-1}$ blood treatments, as well as between 2.5 t $ha^{-1}$ and 12.5 t $ha^{-1}$ treatments.

On the Total biomass DM means, significant differences ($P < 0.05$) were shown to be between the 25 t $ha^{-1}$ and the negative control, and between 12.5 t $ha^{-1}$ and both the negative control and 0 t $ha^{-1}$ treatment.

Although the differences between the negative control and the 2.5 t $ha^{-1}$ were not statistically significant, all blood treatments gave a mean DM yield at least four times greater than the mean negative control for both shoot and biomass DM (Table 3.6).

**Table 3.6** – Plant growth trial data.

| Treatment | Mean shoot DM (g) | Mean total root & shoot biomass DM (g) |
|---|---|---|
| high blood (25 t $ha^{-1}$) + lime | 2.32 (± 0.49) a b c | 3.22 (± 0.74) a b |
| med blood (12.5 t $ha^{-1}$) + lime | 3.46 (± 0.21) a | 4.77 (± 0.50) a b |
| low blood (2.5 t $ha^{-1}$) + lime | 1.48 (± 0.16) b | 2.93 (± 0.51) a b c |
| no blood (0 t $ha^{-1}$) + lime | 0.57 (± 0.01) c | 2.43 (± 0.51) a c |
| no blood + no lime | 0.35 (± 0.05) c | 0.73 (± 0.05) c |

Each value shown is the mean of four replicates ± sem.
Differing suffix letters denote significant differences ($P < 0.05$)

### 3.5 Discussion

The blood waste was inoculated with *S. poona* due to the regular isolation of the bacterium in poultry, for which *Salmonella* spp. has been reported to be as high as 29% in retail chicken (Harrison et al., 2001). *E. coli* O157 has also been found in both broiler flocks (Schouten et al., 2005) and retail chicken meat (Akkaya et al., 2006). It is therefore expected that both these pathogens could be present within poultry blood waste. Furthermore, inoculating the waste with a *lux*-marked strain of *E. coli* O157 gave further information regarding the effect of lime addition on pathogens without the issue of stressed cells entering a viable but non-culturable state. Both organisms are also Gram-negative, and it is known that increasing the surrounding pH of Gram-negative pathogens leads to disruption of their cytoplasmic membrane hence leakage of intracellular fluids thus rapid cell death (Mendonca et al., 1994). As other pathogens commonly found in abattoir waste such as *Campylobacter* spp. (Fransen et al., 1996; Wempe et al., 1983) and *Shigella* spp. (Ezeronye and Ubalua, 2005) are also Gram-negative, our findings indicate that it is likely that lime application will be effective against these also.

Alkaline treatments of biological wastes have been routinely used in the wastewater and sludge treatment industry for a considerable length of time, as it is a cost effective method of decreasing the pathogen load contained within the waste (Avery et al., 2009; Boost and Poon, 1998; Smith, 1996).

Whilst Buncic et al. (2002) tested poultry blood for these pathogens (and others) they failed to isolate either of these bacteria. It was thought that this was due to having low initial numbers of pathogens (below the methods' detection limits) as a dilution effect of having many negative animals' blood mixed in with those who shed the bacteria. Typically a broiler produces just 25g of blood (Buncic et al., 2002), the effects of which are easily diluted within the hundreds of litres of blood waste typically produced at a poultry slaughterhouse per day. Whilst blood may not intrinsically have high levels of pathogens at the point of slaughter, other abattoir wastes, such as gastro-intestinal tract contents, intermittently do have high levels of these bacteria such as *E. coli* O157:H7 (Buncic et al., 2002; Laven et al., 2003) and *Salmonella* spp. (Furrer et al., 1993). Thus cross-contamination of blood waste with other forms of abattoir wastes would provide favourable conditions for pathogen growth (Avery et al., 2009), and necessitates the requirement for the safe handling of blood waste.

Current EU legislation means that a large proportion of poultry abattoir waste has to be disposed of via rendering or incineration on the basis of increased biosecurity. However, if an effective treatment method for such waste could be found, this would merit changes in regulations to accommodate simpler, cost-effective solutions for treatment prior to land-application. This study highlights how lime treatment can be used as an effective method for reduction of both *Salmonella* spp. and *E. coli* O157 from abattoir wastes. This, along with the 3 $\log_{10}$ decreases in the TVC, show that these reductions are likely to be applicable to a range of other enteric pathogens.

Lime-treatment and subsequent land-application of poultry blood could present significant financial savings to both poultry processors by decreasing their waste disposal costs and to farmers who by spreading this waste mixture on grassland would save on both lime and fertiliser costs whilst gaining productivity.

The grass growth trials show that injection of lime-treated blood into the grass sward gives an increase in productivity. The DM yield for both shoot and total biomass was four fold higher than the untreated sward. From the data presented it can be seen that both CaO and $Ca(OH)_2$ are both highly effective treatments for poultry blood waste, killing the large numbers of pathogen bacteria added to the waste in the experiment. Combined with the preliminary research conducted, these results show that the optimum level for treatment will be around the 2.5% level, as the 1% treatments (data not shown) was not consistently effective in killing either of the pathogens consistently over the two hours tested. Due to the great volume of work that this trial requires, only one type of Calcium compound was tested at a time, thus the work was repeated in triplicate to ensure accuracy.

It is plausible that levels of the more reactive CaO could be reduced as no *Salmonella* spp. were isolated from any of the treatments over the whole experiment and only one replicate at time 0 for *E. coli* O157. $Ca(OH)_2$ would appear not to have the ability to be reduced by much due to the continued isolation of *E. coli* O157 for over 40 minutes in one replicate. In addition, it is plausible that in a laboratory the mixing technique was more effective than would be possible under industrial conditions, thus perhaps the recommendation for using this would include a time period after treatment prior to application to land, in order to allow the sample to be fully homogenised and thus the lime to work fully. $CaCO_3$ was simply not effective (data not shown) at all in reducing the pathogenic load, due to it's insolubility in the waste and inability to raise the pH.

Avery et al. (2009) showed that blood wastes gave greater pH increases for a given dose than mixed wastes (intestinal contents, etc.). Additionally, the significant amount of water used at the killing points and the subsequent stages of the slaughter process means that the blood waste is far more dilute than the pure blood which means that the lime is able to work more effectively upon any pathogens present.

Whilst understanding that laboratory findings cannot be directly extrapolated to the agricultural environment, our work does corroborate with previous studies (Abbas and Choudhary 1995; Avery et al. 2009; Petroczki, 2004). It would be desirable that

the neutralizing value (NV) of the treated sludge should be confirmed in a larger scale trial. Limes are typically given a NV which is a figure that represents the percentage of the effect obtained if pure CaO were to be used (Parkinson, 2003). However, the addition of blood will reduce this value, and should be tested to determine figures for both CaO and $Ca(OH)_2$ for use by farmers.

From an industry perspective, the financial aspects of the two treatments must also be considered. Whilst CaO seems to be slightly more effective in killing pathogens than $Ca(OH)_2$, it is also more expensive, with typical current bulk prices of £80-£100 per tonne which is 5-10% greater than $Ca(OH)_2$ (Tarmac Buxton Lime and Cement, *personal communication*). However, exact costs would vary depending on distance from the producer (approximately 25% of the total cost) and the fineness of the product; the more finely ground the CaO product is, the more energy required for grinding, and thus the more expensive it is. All of this obviously will also have a part to play in the decision of which chemical to use and how it is used (time left for, mixing required etc).

Effective mixing of the blood waste and lime is crucial to the success of this treatment. After a few minutes the powdered lime starts to fall out of suspension and collects at the bottom of the centrifuge tubes. Thorough mixing at time 0 ensured that the lime had the optimum conditions in which to be effective against the pathogens from the beginning, and constant lateral mixing by the incubated shaker mimicked mixing that could occur during transportation.

Blood clots could be a potential problem with the treatment, as theoretically large clots could harbour pathogenic bacteria and inhibit the effectiveness of the lime treatment. The pumping action from the abattoir's storage facilities plus the way in which the lime is mixed into the waste in practice, must take this into account in order to prevent this problem. Additionally, it is probable that more coarse mixes will need greater mixing or perhaps higher doses of lime to ensure the required lethality to pathogens.

From the results found in this study, an industrial scale would be necessary to confirm the findings. Variables such as lime granular size verses the cost should be

taken into account in order to find the best solution from both a practical and economic perspective. Specific attention should also be given to the issue of homogenising the blood waste to ensure that large blood clots do not harbour pathogenic bacteria.

### 3.6 Conclusions

This study shows that lime treatment of poultry abattoir blood waste using either CaO or $Ca(OH)_2$ is a viable solution for reducing pathogen loads prior to disposal and application of the waste. When added to agricultural land the lime-treated waste gives greater productivity from grasslands, which may thus give a safe and economical method of utilising nutrients that may be otherwise lost to production systems.

Prior to adoption, further studies should be undertaken to ensure that these methods work in practice using the large volumes of waste generated by poultry abattoirs. If effective, this may suggest that the EU ABPR (1774/2002) should be amended to include this as an option available to the poultry industry for treating blood waste prior to disposal.

### Acknowledgements

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# Chapter 4

## Microbiological quality of chicken wings damaged on the farm or in the processing plant[1]

[1]This chapter has been published in Food Microbiology:
Malpass, M.C., Williams, A.P., Jones, D.L. and Omed, H.M. 2010. Microbiological quality of chicken wings damaged on the farm or in the processing plant. Food Microbiology, 27:521-525.

## 4.1 Abstract

Selling of damaged chicken wings (those with bone protrusion) for human consumption is prohibited in the European Union on the grounds of possible risks to human health arising from microbial contamination. Standard food industry tests were used to assess different categories of chicken wings (undamaged, farm damaged and factory damaged; $n$ = 264) for, coliforms, Enterobacteriaceae, total viable counts, *Pseudomonas* spp., *Staphylococcus aureus* and *Salmonella* spp.. No significant differences in bacterial numbers existed among wings belonging to the three categories. Only low numbers of bacteria were found throughout, and 97% of all results would pass the standards of a leading UK retailer. These results were strengthened by a longitudinal survey of wing breakage, which showed almost all wing puncturing occurred during the de-feathering process, limiting the likelihood of microbial contamination. Combined, these results indicate there is no increased health risk from consumption of damaged, compared to undamaged, chicken wings. The existing imposed regulations may therefore be an unnecessary burden on the poultry industry.

## 4.2 Introduction

Damage to chicken wings (bone protrusion) represents a significant problem in the poultry industry as such wings are prohibited from passing though a production line. The whole of the damaged wing must therefore be manually trimmed from the carcass, preferably following evisceration to minimise the risk of contamination of exposed meat (Anon, 2007). At a typical large poultry slaughterhouse, processing approximately 40,000 broilers per day, the overall cost arising from damaged wings has been estimated at £65,000 per annum due to reduced productivity, loss of yield, and significant overheads arising from inspection and disposal costs (D. Broxton, personal communication).

Chicken wings can be visually categorised into the following three groups: normal or undamaged (N), farm damaged (FD) (damaged before reaching the processing plant, showing blood retention and/or bruising around the joint, usually protruding bones), and factory machine damaged (MD) (bone protruding through the skin but absence of blood around the joint or under the skin). This categorisation is based on the notion that blood retention arises due to damage occurring pre-slaughter (FD), whereas the lack of blood indicates that the carcass has already been bled before the damage occurs (MD). Most farm damage is thought to occur during the hand catching stage, and is highly dependent upon the technique of the catching team (Kettlewell and Turner, 1985). Machine damage is most likely to occur during the de-feathering process (as the kinetic energy used to remove the feathers is considerable) and not during the shackling process (Jones and Satterlee, 1997; Jones et al., 1998a, 1998b).

Chicken skin is naturally contaminated with microbes from grazing and processing (Uyttendaele et al., 1997; Avens et al., 2002). It is hypothesized that if wings are damaged before reaching the processing plant (i.e. on-farm or during transport), there are greater opportunities for bacteria to enter the wing through the broken skin. Further, tissue contamination may also occur within the processing plant scald tank where there may be high levels of bacterial contamination (Geornaras et al., 1996, 1998; Cason et al., 2000; Cansian et al., 2005). Therefore, it is implicitly believed that damaged wings will carry an unacceptably high microbiological load both on the

exterior and inside of the meat. Indeed, selling damaged wings is prohibited in the EU on the grounds of potential microbial contamination (Anon, 2007). However, as the majority of MD wings are damaged in the plucking machine (i.e. after the scald tank), this would lessen the possibility of microorganisms entering the wing. We therefore hypothesise that the microbial load of FD wings will be greater than MD wings.

The first aim of this work was to undertake a longitudinal survey of broiler wings, following the same cohort of birds from catching through to post-evisceration to determine the point where maximum wing damage occurs. The second aim was to determine whether damaged wings possessed different microbial loads to undamaged wings and to ascertain whether damaged wings are justifiably deemed unfit for human consumption. The study included determining the numbers (or presence) of specific foodborne pathogens, indicators of faecal contamination, and bacteria associated with microbial spoilage or overall microbiological quality.

## 4.3 Materials and methods

### 4.3.1 Longitudinal surveys of wing damage

Studies were undertaken to determine the critical points at which damage to chicken wings occurs during the rearing to processing life cycle. These were undertaken on five farms supplying a large commercial chicken plant processing approximately 40,000 birds per day. All farms were independent of each other and were from different production sites to ensure heterogeneity in farms conditions. Tagged transport modules ($n$ = 5; 706 birds in total) of caught broilers were manually checked for physical wing damage prior to the module being loaded onto transportation trucks (post catching at farm). The same catching team was present for all of the sample modules, though they were not made aware as to the reasons for the module checking. Catching was conducted by hand, with multiple birds per catcher's hand. Transport distances from farm to plant ranged from 5–35 km. The same module was then checked in the factory lairage after transportation and unloading. Both of these checks were conducted by removing every bird from the module, feeling the wing joint for dislocation, and visually assessing it for blood and/or

protruding bones. The numbers of birds per module, total numbers of pullets and the occurrence of leg damage were also recorded.

Each module was separated and processed separately on the production line, using markers and a 50 shackle gap for clear identification. These broilers were then followed through the slaughter and processing line, with surveys of damaged wing incidence being conducted after the neck cutter, plucking machine and post-evisceration. Surveys were conducted using mechanical counters to ensure accuracy (one each for pre- and post-mortem damage).

4.3.2 Microbiological quality of wings

Samples were collected at various times from September 2006 through to November 2008 at the same large commercial chicken plant where the longitudinal study was undertaken. Wings were collected randomly post carcass evisceration (prior to entering the air-chiller section) to give the maximum opportunity for cross-contamination from the scald tank (Geornaras et al., 1996, 1998; Cason et al., 2000; Cansian et al., 2005), plucking machine (Berrang and Dickens, 2000; Rasschaert et al., 2007a), and evisceration (Corrier et al., 1999). This represents the same point at which poultry inspection assistants (PIA) remove all damaged wings. After collection, the wings were placed inside sterile plastic bags, placed on ice in a cool box and taken to the laboratory for analysis within 1 h of collection. At each independent sampling event, triplicate samples of undamaged, farm damaged, and factory damaged wings were taken for analysis.

4.3.3 Microbiological food quality standards

All the methods employed were based upon food industry microbiological quality standards, specifically those detailed in the Marks and Spencer Microbiology Methods Manual (Anon, 2004), which are based on Campden and Chorleywood Food Research Association accredited methods (Campden BRI, Chipping Campden, UK). It was decided to base the wing testing on Marks and Spencer's (M&S) stringent Food Quality Standards (Table 4.1) as these are widely regarded to be amongst the highest within the UK food industry (D. Broxton, personal communication). Depending on its microbiological status, raw chicken can be categorised into one of three classifications within the standards; namely,

'acceptable', 'borderline', or 'unacceptable'. Chicken categorised both in the 'acceptable' and 'borderline' classification are regarded of sufficiently high microbiological quality to be deemed fit for human consumption.

**Table 4.1** – Marks & Spencer microbiological Food Quality Standards for raw chicken (numbers are measured in $\log_{10}$ CFU $g^{-1}$ sample).

| Test | Agar used | Acceptable no. | Borderline no. | Unacceptable no. |
|---|---|---|---|---|
| Coliforms | VRBA | $< 2$ | $2 - 4$ | $> 4$ |
| Enterobacteriaceae | VRBGA | $< 4$ | $4 - 7$ | $> 7$ |
| Total viable counts | Standard plate count agar | $< 5$ | $5 - 7$ | $> 7$ |
| *Pseudomonas* spp. | *Pseudomonas* agar | $< 4$ | $4 - 6$ | $> 6$ |
| *S. aureus* | Baird-Parker Medium | $< 2$ | $2 - 3$ | $> 3$ |
| *Salmonella* spp. | XLD and BGA | Absent in 25 g | Absent in 25 g | Present in 25 g |

### 4.3.4 Enumeration of bacteria

Unless otherwise stated, all assay ingredients were sourced from Oxoid Ltd. (Basingstoke, UK). For the five bacterial counts tests, the sample preparation was as follows: Briefly, a composite (10 g) sample containing skin, muscle, cartilage, bone fragments and any traces of feather left on the wing after plucking were aseptically recovered from each wing ($n$ = 264; 88 from each category) using sterile scissors. The sample was firstly cut from the muscle around the humerus and then continued around the joint to the muscle adjoining the radius and ulna. This method ensured the joint was thoroughly opened and that the surrounding meat (therefore the areas most likely to be contaminated) was included in the sample. Maximum Recovery Diluent (90 ml; MRD) was then added to the sample before stomaching in a Seward 400 circulator machine (Seward Ltd., Worthing, UK) at 230 rev $min^{-1}$ for 30 s (Anon, 2004). Serial dilutions were then prepared from the stomached sample for the following analysis, with all solutions plated in triplicate:

For coliform bacteria and Enterobacteriaceae counts, 1 ml of prepared samples was pipetted into sterile petri-dishes before being overlaid with molten Violet Red Bile Agar and Violet Red Bile Glucose Agar, respectively; then mixed by agitation. Total viable counts (TVC) were determined via placing 0.5 ml aliquots of serially diluted samples into sterile petri-dishes then overlaying with molten standard plate count. Once set, all plates were subsequently incubated at 37°C before enumeration of

characteristic colonies after 24 h. To determine numbers of *Pseudomonas* spp., 0.5 ml aliquots of serially diluted samples were pipetted onto solidified *Pseudomonas* agar (containing additional CFC supplement X3754H) and were incubated at 25°C for 48 h. Rehydrated Oxidase reagent (Fluka Chemie GmbH, Germany) was subsequently pipetted onto the agar and the colonies counted. *Staphylococcus aureus* numbers were determined by plating 0.5 ml aliquots of serially diluted samples onto solidified Baird-Parker medium containing egg yolk tellurite emulsion (10% v/v) and enumeration after incubation at 37°C for 48 h (Oxoid, 2010a). Colonies were confirmed as *S. aureus* with an Oxoid latex agglutination kit (DR0595A).

To test for the presence of *Salmonella* spp., 25g samples were removed from the same wings described previously and placed in 225 ml of Buffered Peptone Water, before stomaching as described previously. The mix was then transferred to a sealed sterile container and incubated at 37°C for 18 h. Aliquots of the enriched sample were then pipetted into either 10 ml of Rappaport-Vassiliadis Medium or 10 ml of Muller Kaufmann tetrathionate novobiocin broth, then incubated for 24 h at 41.5°C and 37°C, respectively. One 10 µl loopful of each of these selective media was sub-cultured onto xylose lysine deoxycholate agar (XLD) and Brilliant Green Agar (BGA), then incubated at 37°C for 24 h. Five presumptive colonies were selected from each media plate and streaked onto nutrient agar and incubated at 37°C for 24 h to remove any remnants of selective media. These purified colonies were then emulsified in 2.5 ml of sterile saline solution, mixed, pipetted into individual wells of an Oxoid Microbact™ 12A biochemical test strip, and incubated at 35°C for 24 h. Well results were read in accordance with the kit's instructions and the interpretation performed by the Microbact™ Computer Aided Identification Package (Oxoid).

### 4.3.5 Data analysis

All bacterial counts were $\log_{10}$ transformed prior to statistical analysis. The dataset was analysed using SPSS 12.0 (SPSS Inc., Chicago, IL). Statistical differences between the microbiological quality of the three wing categories was undertaken by ANOVA with Tukey pair-wise comparison and with $P = 0.05$ used as the statistical threshold for significance. An equal distribution of variances was confirmed via a Levene's test.

## 4.4 Results

### 4.4.1 Longitudinal survey

The results from the longitudinal survey are presented in Table 4.2. Careful physical checking of live birds showed that even when pre-mortem damage occurred, bones were very rarely dislocated during catching and transportation (0.3% of all birds), and even in those cases no bones were found to be broken or protruding through the skin. Whilst a small percentage of damage occurred as a result of dislocation (0.7% of all birds; observed to occur from on-shackle flapping, where wings were hanging down and thus passing through the neck cutting blades), it is important to highlight that this was usually just the wingtip and not through the flesh.

**Table 4.2** – Occurrence of wing damage in a longitudinal survey of chicken from the rearing to the processing life cycle ($n$ = 706 birds from five independent transport modules).

| Sampling location | % damaged birds |
|---|---|
| Post catching | 0.0 |
| Post transport | 0.3 |
| Post neck cutter | 1.0 |
| Post plucking | 12.6 |
| Post evisceration | 13.0 |
| | |
| Source of damage of post evisceration sample | % damaged birds |
| Pre mortem (bloodied and broken) | 3.8 |
| Neck cutter damaged | 0.7 |
| Post mortem (broken skin) | 8.5 |

This survey showed that the vast majority of damage (12% of all birds) occurred between the neck cutter and the PIA point after the plucking machine (Table 2). As the scald-tank provides limited opportunities for such damage to occur, it indicated that the plucking machine was the primary cause of the damage. After this point the evisceration process is conducted, which includes a number of shackle and carcass transfers, offal removal and carcass washing. However, this process only gave a small increase (0.4% of all birds) in damaged wing incidence. Regression analysis

found no statistically significant correlation between the incidence of either pre- or post-mortem damage and either the number of birds per module, total number of pullets, leg damage or farm damage ($P > 0.05$; data not shown). The same applied between these factors and total damage post evisceration (including neck-cutter damage; $P > 0.05$). However, a high degree of correlation ($r > 0.9$) was found between damage found in the lairage and both post-mortem damage and the total incidence of damage ($P < 0.05$).

4.4.2 Microbiological quality

The results of the bacterial enumeration tests for the five microbiological food quality indicator groups are shown in Table 4.3. Undamaged chicken wings tested positive for all five microbiological food quality indicators (coliforms, Enterobacteriaceae, TVC, *Pseudomonas* spp. and *S. aureus*). However, statistical analysis revealed no significant difference in bacterial population numbers between these and either the farm or machine damaged wings for all five microbiological food quality indicators ($P > 0.05$).

**Table 4.3** – Counts of food quality bacterial indicators ($\log_{10}$ CFU $g^{-1}$ sample) recovered from undamaged (Normal), farm damaged and factory machine damaged chicken wings.

| **Test** | Normal | Farm damaged | Machine damaged |
|---|---|---|---|
| Coliforms | 2.29 ± 0.71 | 2.46 ± 0.72 | 2.46 ± 0.78 |
| Enterobacteriaceae | 2.39 ± 0.62 | 2.58 ± 0.69 | 2.57 ± 0.71 |
| Total viable counts | 3.75 ± 0.55 | 3.77 ± 0.50 | 3.77 ± 0.53 |
| *Pseudomonas* spp. | 2.50 ± 0.72 | 2.60 ± 0.56 | 2.41 ± 0.80 |
| *S. aureus* | 1.75 ± 0.45 | 1.78 ± 0.39 | 1.76 ± 0.52 |

Numbers represent $\log_{10}$ transformed means ± standard deviation ($n = 264$, 88 from each category) All mean values on the same line are not significantly different ($P > 0.05$).

There was no significant difference in microbial load among the different categories of wing throughout all five numerical tests (Table 4.3). A total of 1584 tests (six per wing) were conducted on the wings collected ($n$ = 264 wings, 88 from each category). Of these, only eight wings (3.0%) would be rejected on the basis of their microbiological quality according to the M&S Food Quality Standards (Table 4.4).

Overall, the results show no discernable difference among the microbiological qualities of the three different wing categories, with a similar number of wings from each category in every classification (Table 4.4). Indeed, there was similar distribution of results for each quantitative tests performed among all three wing categories (Table 4.5).

**Table 4.4** – Classification of undamaged (Normal), farm damaged and factory machine damaged chicken wings according to the Marks & Spencer microbiological Food Quality Standards for raw chicken ($n$ = 264, 88 from each category).

| Classification | Wing classification | Number of wings (%) |
|---|---|---|
| **Acceptable** | **Total** | **73 (27.7)** |
| | Normal | 26 (9.9) |
| | Damaged | 47 (17.8) |
| | - Farm damaged | 24 (9.1) |
| | - Machine damaged | 23 (8.7) |
| **Borderline** | **Total** | **183 (69.3)** |
| | Normal | 60 (22.7) |
| | Damaged | 123 (46.6) |
| | - Farm damaged | 63 (23.9) |
| | - Machine damaged | 60 (22.7) |
| **Unacceptable** | **Total** | **8 (3.0)** |
| | Normal | 2 (0.7) |
| | Damaged | 6 (2.3) |
| | - Farm damaged | 1 (0.4) |
| | - Machine damaged | 5 (1.9) |

As the *Salmonella* test was an enrichment process, our results only indicate the presence or absence of the organism. Out of the 264 wings tested, only two of these (0.8%) tested positive (one N, one MD) following biochemical analysis with an Oxoid Microbact™ 12A kit.

**Table 4.5** – Percentage (%) of undamaged (Normal), farm damaged and factory machine damaged chicken wings conforming to different categories of the Marks & Spencer microbiological Food Quality Standards for raw chicken for various microbiological tests.

| | Normal | | | Farm damaged | | | Machine damaged | | |
|---|---|---|---|---|---|---|---|---|---|
| Test | Acceptable | Borderline | Unacceptable | Acceptable | Borderline | Unacceptable | Acceptable | Borderline | Unacceptable |
| Coliforms | 12.5 | 20.8 | 0.0 | 11.0 | 22.4 | 0.0 | 9.9 | 22.7 | 0.8 |
| Enterobacteriaceae | 32.6 | 0.8 | 0.0 | 32.2 | 1.1 | 0.0 | 31.4 | 1.9 | 0.0 |
| Total viable | 32.6 | 0.8 | 0.0 | 33.0 | 0.4 | 0.0 | 33.0 | 0.4 | 0.0 |
| *Pseudomonas* spp. | 32.9 | 0.4 | 0.0 | 32.9 | 0.4 | 0.0 | 32.9 | 0.4 | 0.0 |
| *S. aureus* | 28.8 | 4.2 | 0.4 | 29.2 | 3.8 | 0.4 | 28.4 | 3.8 | 1.1 |
| *Salmonella* spp. | 32.9 | 0.0 | 0.4 | 33.3 | 0.0 | 0.0 | 32.9 | 0.0 | 0.4 |

Percentages represented as a total of the wings included in that test ($n = 264$, 88 from each category)

**4.5 Discussion**

Based upon industry standards, our study found no compelling evidence to suggest that either farm or factory damaged chicken wings should be categorised as unfit for human consumption on the grounds of their microbiological status. We attribute this to our finding that the skin puncturing and major damage occurs after the scald tank during the de-feathering process, even for farm damaged wings. Thus there are few opportunities for bacterial pathogens to enter the opening in the wing, even though the bruising may have occurred prior to this. Our results therefore imply that the risks of foodborne illness associated with consumption of damaged wings are equal to those associated with undamaged wings.

The lack of skin puncturing damage during catching and transportation also directly implies that opportunities for bacteria to enter the skin and contaminate underlying flesh are severely limited. The results indicate that in both types of damage, puncturing primarily occurs during the de-feathering process. This is a significant finding as it means that faecal contamination during transportation, lairage or when the birds are shackled is no greater than that of normal wings/carcasses (Northcutt et al., 2003). Likewise, the bacteria contained in the scald tank "soup" (Cansian et al., 2005) do not get the opportunity to contaminate the underlying chicken meat. Similarly, bacteria in the blood and crop contents are prevented from passing through the skin during the neck cutting and bleeding process. During evisceration, intestinal contents could potentially leak into the opening in the wing skin, leading to contamination. However, within modern automated processing plants the risk from this remains very low for two reasons. Firstly, as careful evisceration is critical to the microbiological quality of the end product (as highlighted on the HACCP drawn up by each factory) the machine settings are highly optimized so few, if any, of the viscera rupture. Secondly, in the rare case of viscera rupture, according to our knowledge and observations, the whole carcass is graded out of the production line and deemed not fit for human consumption by trained PIAs. Based upon this and the results presented here, it can therefore be concluded that in a well-run processing factory, this form or type of wing contamination does not occur.

Overall, < 1% of samples tested positive for *Salmonella* which is lower than the 6-29% prevalence of the bacterium found on raw chicken products in previous studies

(Harrison et al., 2001; Jørgensen et al., 2002; Meldrum et al., 2005b). This lower incidence is probably due to the high level of biosecurity (e.g. personnel and feed) operating on the farms from where the tested chickens originated.

The findings of this study could have notable financial implications for the chicken industry in terms of cost savings. Firstly, if damaged wings are graded out during carcass cut-up, one less trained PIA (employed at higher rates of pay than regular factory employees) is needed as there are already operatives grading wings during carcass portioning. Secondly, many automated carcass cut-up machines use both wings in order to remove the breast fillets efficiently; therefore those without two wings must go down a manual fillet removal line with additional operators and costs. There could also be a significant saving on (waste) disposal costs if the regulations can be altered to permit the sale of damaged wings. Markets for the sale of damaged wings could include processed meat manufactures and soup makers, generating additional revenue to poultry processors.

Further research is needed in this area of food microbiology and to extend the study to different catching teams and processing plants. However, in conclusion, this study found no major difference in the microbial loads between normal, farm damaged or factory damaged chicken wings. The premise that damaged chicken wings are microbiologically spoiled and consumption poses a risk to human health should therefore be reconsidered.

### 4.6 Acknowledgements

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# Chapter 5

## Investigation into the Incidence of Chicken Wing Damage across Different UK Broiler Production Systems

## 5.1 Abstract

Chicken wing damage is an important production issue as Meat Hygiene Service (MHS) regulations prevent damaged wing from being sold for human consumption. Broiler pre-mortem wing damage is also an area of concern on welfare grounds. Pre and post mortem damage was assessed at two processing factories on broilers reared to conventional standards at 3.8 kg and 2.2 kg target bodyweight, and to the RSPCA's Freedom Foods (FF) standards at 2.2 kg. Significant differences in the incidence of wing damage were recorded between all groups, with the heavy (3.8 kg) conventional broilers averaging 3.4% pre and 7.0% post-mortem wing damage. By contrast, lighter, conventionally reared birds had an incidence of 1.0% and 4.0% pre and post-mortem. However, FF standards broilers had a pre-mortem wing damage of 0.2% and a post-mortem incidence of 1.5%. As pre-mortem damage has a tangible link to the pain suffered by live birds, we conclude that the enriched production system conditions in FF production can be observed to have a significant positive effect upon bird welfare.

## 5.2 Introduction

Damage to chicken wings (bone protrusion) currently poses a significant economic loss for poultry producers as existing legislation (Anon, 2007) requires any damaged wings to be manually removed as they are not regarded of sufficient quality for human consumption on the grounds of possible microbial contamination (Anon, 2007). In addition to the loss of income, this incurs further financial burdens as producers must dispose of them as Category 3 hazardous waste. Damaged wings are typically categorised as "farm-damaged" (bloodied and broken) or "machine-damaged" (just broken) on the notion that blood retention occurs due to damage occurring prior to slaughter, whereas the lack of blood indicates that damage occurred after slaughter and the carcass has already been bled prior to damage occurring (Knowles and Broom, 1990; Malpass et al., 2010).

However, the term "farm-damaged" is slightly inaccurate in so far that although some damage occurs as a result of poor catching methods (Kettlewell and Turner, 1985), a significant proportion of damage occurs at the processing plant during shackling and prior to stunning, with both damage and haemorrhaging resulting from wing flapping during this time (Gregory *et al.*, 1989; Jones and Satterlee, 1997; Jones et al., 1998a, 1998b). Similarly, Griffith (1985) was able to proportion leg damage by ageing bruises, and attributed 25% to those sustained in the time prior to catching, 40% to catching and crating, and a further 30% to the time after slaughterhouse arrival. Thus, it is perhaps more accurate to think of the categories as pre-mortem (bloodied and broken) and post-mortem (just broken) damage.

Whilst current legislation views damaged wings from a food safety perspective, any wing damage, particularly bone breakage itself, is also likely to incur considerable amounts of pain to a live bird (Knowles and Broom, 1990). There is increasing emphasis on enhancing animal welfare in the poultry industry, from both consumers and policy-makers. It is therefore desirable from both an animal welfare perspective and from an economic perspective to minimise the occurrence of wing damage within poultry production and processing.

There is widespread consensus that a large proportion of European consumers are concerned by the concept of animals suffering or being mistreated during food

production (Chilton et al., 2006). In the UK, this has been reported as being as high as 86% (Bennett, 1997). Combined with studies that have shown consumers' willingness to pay for increased and/or guaranteed levels of welfare (Bennett, 1997; Chilton et al., 2006), this has led to the introduction of a number of market-led initiatives. Some of these schemes have been set up by retailers, others by producer organisations in association with animal welfare organisations such as the RSPCA's Freedom Food scheme (RSPCA, 2008) that are run by Non-Governmental Organisations or charities (Vessier, et al., 2008).

A specific requirement of Freedom Food standard broiler production is the availability of environmental enrichment such as perches and straw bales (RSPCA, 2008) to enable birds to exhibit their natural behaviour, similar to that shown by their wild Jungle-Fowl relatives, who roost on tree branches in their natural environment (Fiscus-LeVan et al., 2000; Pettit-Riley and Estevez, 2001). Broilers have been shown to prefer areas of a rearing shed with objects in them compared to bare ground (Leone et al., 2007), and thus straw bales add to this environmental richness within a shed, decreasing the localised stocking density, which in turn gives other benefits such as improved localised litter quality.

Additionally, aggressive behaviours in chickens have been shown to decline with the availability of perches (Cordiner and Savory, 2000). In addition perches can decrease leg deformations, the incidence and severity of leg problems in broilers (Fiscus-LeVan et al., 2000; Pettit-Riley and Estevez, 2001) and decrease the incidence of leg deformations through increased activity, especially in young birds (Haye and Simons, 1978; Kestin et al., 1992). Similarly, it has been suggested that wing and breast muscles are also exercised during perching through getting on and off, and balancing on the perch, resulting in greater wing strength (Fiscus-LeVan et al., 2000).

Certain factors have been identified previously as increasing the incidence of wing damage and bruising, such as increased live weight (Griffiths and Nairns, 1984; Kestin et al., 1992; Mayes, 1980). In the UK the consumption of broiler meat has grown from an estimated 5.5 kg per capita per year in 1961 to an estimated 20.49 kg per capita per year in 2008 (FAOSTAT, 2010). Additionally, consumer preferences

have changed, with increasing demands for high value cuts such as breast meat (Rogers, 1992). To meet both of these demands the broiler industry has expanded in terms of numbers of birds (FAO 2009) and increased the live-weight at slaughter (Saleh et al., 2004), as larger birds produce larger high-value cuts (Rogers, 1992). The aim of this experiment is to investigate the incidence of both pre and post mortem chicken wing damage between different production systems.

**5.3 Materials and methods**

A previous study (Malpass et al., 2010) ascertained that very limited protruding bone damage (0.3%) occurred in birds arriving at an abattoir, and thus this research focused on wing damage occurring within the factory.

Sampling was conducted at two factory locations in order to investigate three different production systems. Firstly were large broilers (3.8 kg) reared under conventional Assured Chicken Production (ACP) conditions, at poultry abattoir A, referred to in this study as Conventional A (CA). The second production system studied was standard broilers (2.2 kg) in conventional ACP conditions at abattoir B (CB). The final sample comprised birds reared under RSPCA Freedom Foods standards (FF), also at a 2.2kg blueprint weight, slaughtered at abattoir B. Further details of the factors relating to these production systems are shown in table 5.1.

**Table 5.1** – Factors relating to the differences between the tested poultry production systems.

| | Production System | | |
|---|---|---|---|
| | Conventional - Factory A | Conventional – Factory B | Freedom Foods - Factory B |
| Blueprint slaughter weight (kg) | 3.8 | 2.2 | 2.2 |
| Age at slaughter (days) | 53 | 38 | 51 |
| Breed | Ross 308 | Ross 308 | Hubbard JA57 crosses |
| Sex | Cockerel | Pullets | As Hatched |

The selected two abattoirs were chosen as they are both owned by the same broiler integrator that supplies both processing plants with birds that are managed by their own staff and have the same common feed supply, which therefore minimises raw material variation as far as possible. However, whilst both conventional sets of birds were fed on the same series of diets, the Freedom Foods' birds are fed on lower density formulations to ensure the <45g/bird/day maximum growth rate without restricting the birds' access to feed.

Broiler breed was the same (Ross 308) for both conventional types of broilers, whereas a different, slower growing breed (Hubbard JA57) was used in all Freedom Foods rearing systems. Conventional A birds were predominantly male, CB predominantly female, whereas FF birds are grown as hatched and therefore are a mixture of cockerels and pullets.

### 5.3.1 Sampling technique

All broiler carcasses were examined in the same manner for wing damage directly after the plucking machines. Each batch of birds from the different production systems was separated by a long (> 200) shackle gap for clear identification. Examinations were conducted using three mechanical counters to ensure accuracy (one each for pre-mortem damage, post-mortem damage and empty shackles). Different batches were sampled over a number of different days. A typical one vehicle batch contains 3,000 to 3,500 broilers depending on average bodyweight and climatic conditions). Total sample size was calculated from knowing the sample period time, factory line speed and number of empty shackles. During any lengthy gaps of empty shackles or production-line stoppages timing was stopped at the passage of the last bird, and a new sample started at the passage of the next bird to ensure accuracy.

### 5.3.2 Data analysis

The data collected in the production system comparison survey were analysed using SPSS 12.0 (SPSS Inc., Chicago, IL). A code was devised for the three categories of wing, with columns for the two types of wing damage, plus production method. Independent samples t-tests were then conducted for both pre- and post-mortem damage to investigate the effect of bird size and production method.

## 5.4 Results

Conventional broilers sampled at abattoir A (CA) had the highest incidence of both pre and post mortem damage at 3.4% and 7.0% respectively (Table 5.2). Whilst post-mortem damage in the conventionally produced birds in abattoir B (CB) was slightly above half the value of CA (4.0%), the pre-mortem damage was more than three times less (1.0%). Independent samples t-tests for both pre and post-mortem damage between CA and CB were both significantly different ($P < 0.01$).

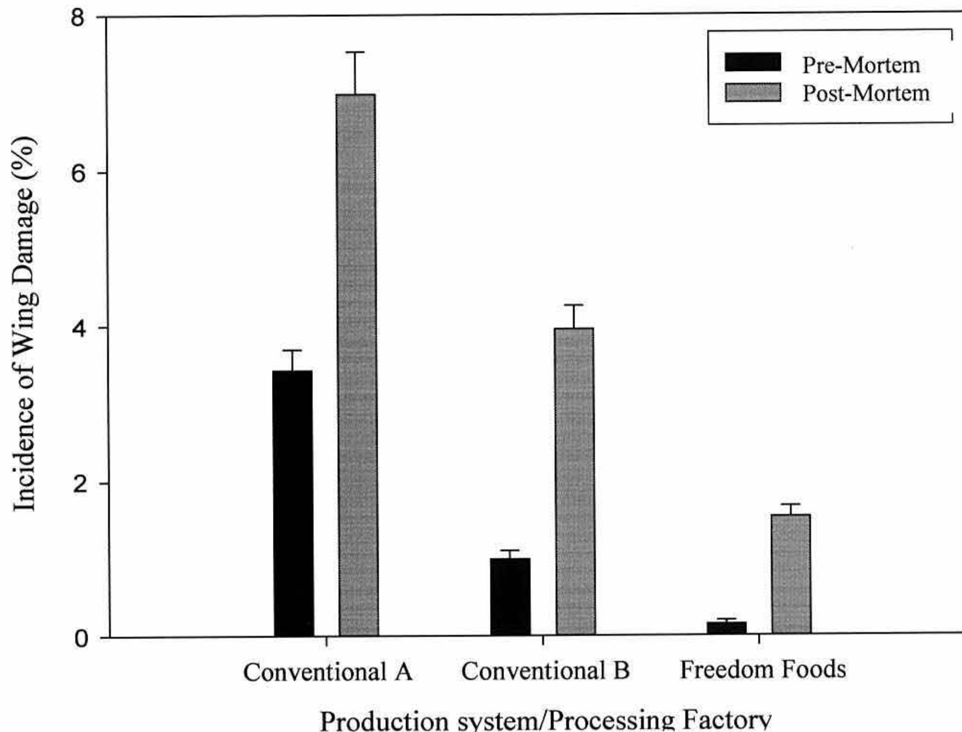
When comparing birds from the same factory, with the same blueprint target weights, the post mortem damage in FF (1.5%) was less than half that of CB and pre-mortem damage for FF (0.2%) was less than a quarter of CB (Figure 5.1). These differences were further analysed using independent samples t-tests and found to be significant for both pre- and post-mortem damage ($P < 0.01$).

**Table 5.2** – Comparison of the effect of different poultry production systems on the incidence of wing damage in birds.

| | Production System | | |
|---|---|---|---|
| | Conventional - Factory A | Conventional – Factory B | Freedom Foods - Factory B |
| Blueprint weight (kg) | 3.8 | 2.2 | 2.2 |
| Total sample size | 25839 | 21943 | 10569 |
| Pre-Mortem damage (%) | 3.43 ± 0.27 | 1.00 ± 0.11 | 0.16 ± 0.04 |
| Post-Mortem damage (%) | 6.99 ± 0.54 | 3.96 ± 0.30 | 1.54 ± 0.13 |

Values Represent Total incidence of damage (%) ± SEM calculated from individual sampling.

**Figure 5.1** – Incidence of wing damage for conventionally reared broilers processed at two different factories and RSPCA Freedom Food standards reared broilers.




Error bars represent + 1 SEM

### 5.5 Discussion

Farm animal welfare is a contentious matter, in which different individuals and areas of the world hold a broad spectrum of views, though globally animal welfare policy development is increasing (Edge and Barnett, 2009). There is no generally accepted definition, nor agreement on what specifically constitutes "good" or "poor" welfare (Chilton et al., 2006).

Some have suggested poor welfare is when its physical health is affected (McGlone, 2001) though others define it as the ability of an animal to cope with it's environment (Broom, 2003) be they hormonal changes or behavioural indicator changes as indicators of welfare (Dawkins, 1990; Duncan and Petherick, 1991; Moberg, 2000).

The origins of increased awareness about farm animal welfare can be traced back to the Brambell report (1965). Although the report had no official link to the E.U. regulations that followed, it was influential in the U.K. and rest of Europe, highlighting some of the poor conditions found at that time and widening the concept of animals' suffering both physically and mentally (Vessier et al., 2008). Indeed, the five freedoms of animal welfare, which are now widely accepted and used, were based on his report.

There was a highly significant difference in the levels of both pre- and post-mortem wing damage between conventionally produced broilers and those reared under RSPCA Freedom Foods standards (Figure 5.1). Whilst the decrease in post-mortem damage is important for production efficiencies, pre-mortem damage decreasing from 1.0% to 0.16% has a major impact upon broiler welfare. Unlike qualitative observational sampling, which potentially varies depending on the assessor's interpretation, this indicator shows an empirical value for the number of birds suffering pain before death.

Importantly, for CB/FF comparison, the same production line was used throughout the weeklong duration of this experiment, sampled typically a few minutes apart. Additionally, despite the different methods employed in production, the blueprint target weight was the same (2.2 kg). This enables these factors to be ruled out as causes of variability in the incidence of wing damage, which indicates that the methods involved in the rearing of broilers (potentially including breed), affects their welfare, not just within the house during rearing, but also during the process of harvesting, transportation and slaughtering.

The FF broilers birds were of the Hubbard JA 57 breed and whilst not specified by the Freedom Foods standards (RSPCA, 2008), the JA 57 is considered to be a slower growing breed (Hubbard, 2009). As FF standards state that feed must be offered to broilers ad libitum and that their growth rate should not exceed 45g per day on average, the FF birds are also fed a lower specification diet than fed to conventional broilers such as the Ross 308 (Ross, 2007a). Both of these factors cannot be ruled out as contributory to the decreased incidence of wing damage. It is highly likely that the slow growth rates and more movement of the birds reared under FF systems would

contribute to healthier and stronger bones and joints compared to the rapid growth and less movement that are associated with conventional systems. Further investigations would be needed in order to attribute the relative importance of each factor.

Furthermore, breed also has an effect upon carcass morphology (Ajayi and Ejiofor, 2009; Grashorn, 2006; Shahin and Elazeem, 2005). After consulting the plucking machine supervisor at factory B it was ascertained that the settings are changed between the types of birds, FF needing the plucking machine sides slightly narrowed and further away from the shackles to account for the different carcass morphology as FF have longer narrower carcasses, resulting from having proportionally larger thighs (Grashorn, 2006). Nevertheless, every load (whether conventional, organic or Freedom Foods) was examined for carcass damage by the employee, and adjustments (if needed) made. It seems then, that the different settings within a factory are not majorly responsible for the differences in the damage incidence. However, if incorrectly set up, plucking machines can cause high levels of wing damage, and furthermore could be one of the factors in the differences observed between CA and CB.

Similarly, both age and sex could have an effect upon wing strength. However, studies have shown that both males and older birds have higher skin puncture strengths (Bilgili et al., 1993), which would mean that CA which process older birds should have the lowest incidence of wing puncturing damage, which is completely at odds with our findings.

Broilers raised under Freedom Foods standards are raised in a seemingly enriched environment, with perches and bales to encourage their natural behaviour (RSPCA, 2008). By contrast, conventional broilers have none of these, indeed the thinning fences, which are of a comparable height to the perches, are sufficient to keep males and females apart. These work well due to the lack of flying behaviour in the conventional birds. Both sets of this respective behaviour have been observed numerous times by the principle investigator during the course of other experiments, which may indicate that the lower damage incidence is related to the increase activity.

Plausibly, it seems that active broilers which regularly use their wings in order to get up onto hay bales and perches have stronger muscles, cartilage and ligaments (Fiscus-LeVan et al., 2000); hence explaining the dramatically lower incidence in damage.

The findings of this study have implications in terms of animal welfare, as it shows that these broilers have stronger limbs and suffer less physical and mental harm as a result of pre-mortem wing damage.

Whilst the reasons behind the differences between CA and CB plausibly may include better staff skills and treatment during catching and hang-on, but more probably in our view, result from CB being younger (by approximately 11 days) and lighter (by around 1.6 kg) birds, and thus being less susceptible to damage during catching, hang-on and from wing flapping when shackled. We hypothesise that the effect of such large muscle mass upon a relatively immature skeleton, means that joints may get over stressed, which then results in dislocation and bone fracture more easily. Further work could include testing the physical properties of conventional and Freedom Foods wings, to determine whether wings on birds bred under Freedom Food standards are indeed physically stronger and thus able to withstand greater forces before breaking. Ideally the two different breeds of broiler should be reared under the same conditions prior to testing in order to ascertain the relative importance of this factor.

The significantly greater incidence of pre-mortem damage in larger birds could be highlighted as a critical area for improvement in broiler welfare. The significant decreases just by having younger, lighter birds may also warrant further investigation, keeping the same methods of catching, hanging and processing to eliminate sources of variation completely. Similarly, increasing bodyweight has been shown to have a detrimental effect upon broiler dead on arrival figures (Nijdam et al., 2004), which could also be included as a factor in further studies as a further indicator of welfare standards.

There has been some concern that the introduction of high welfare standards for broiler (and egg) production, and a subsequent increase in the cost of production, may result in the relocation of production to other countries outside the legislative control of bodies such as the EU (Grethe, 2007). Large broilers are reared primarily for the production of high value, large cuts, particularly large breast fillets, which command a premium price (Rogers, 1992) and thus, in effect, are a luxury item of food. Therefore, one method of improving this aspect of broiler welfare, would be to reduce the demand for these large broilers, which in turn would reduce the pressure to relocate production overseas.

## 5.6 Conclusions

The findings of this study indicate that the production system used for the rearing of broilers has a statistically significant effect on the incidence of both pre- and post-mortem wing damage. Conventionally produced birds at large live weights had the highest incidence of both types of damage. Notably, pre-mortem damage (critical for animal welfare) in birds reared under RSPCA Freedom Foods standards was close to zero. The reasons behind this are likely to include the wing ligaments, muscles and joint cartilage being strengthened by the increased levels of activity in FF birds. This could be as a result of their rearing environment enabling them to exhibit natural behaviours such as perching together with the slow growth rate that reduces the effects on bones and joints. Further research is needed in order to attribute the contribution from other factors such as breed and nutrition. This investigation's findings are evidence that indicates that the higher welfare standards FF birds have during rearing, catching and slaughter results in broilers suffering less pain during the latter part of their lives.

## 5.7 Acknowledgements

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# Chapter 6

## Discussion, Conclusions and Further Work

## 6.1 Introduction

Understanding the microbiology surrounding poultry production is paramount for the economical, efficient and above all, safe production of poultry. Intrinsically linked to this are a number of factors such as welfare, which overlay a framework of additional considerations, some of which (e.g. human social issues) can have a positive or deleterious effect upon the microbiological factors. Taking a 'plough-to-plate' approach to poultry production, the experimental chapters and literature review in this thesis identify a number of ideas and concepts for discussion.

## 6.2 On-Farm Issues

The type of production system employed in rearing broilers can have an effect upon the risk of pathogen introduction. Whilst conventional standards of production have a confined flock that are therefore easier to manage and maintain a strict biosecurity regime, the process of thinning (where a high risk group of the population (catchers; Allen et al., 2008c) enters the shed) potentially infects the flock with pathogens such as *Campylobacter* spp. At the other end of the spectrum, free-range broilers have a greater opportunity for infection from external sources (e.g. wild birds, contaminated ground). Thus a middle ground, such as the RSPCA's Freedom Foods standards, with no thinning and a controllable yet environmentally enriched shed environment, would seem to be a logical and beneficial compromise. As Chapter 5 on physical wing damage has shown, other benefits of Freedom Foods production can be demonstrated in terms of the birds' welfare. Whilst the exact reasons behind this have not been identified, it is likely that a combination of factors play a role in this such as lighter slaughter weights, growth rates, breed and nutrition, together with the presence of bales or perches being in the rearing shed environment (which lead to increased exercise levels and thus stronger wings).

As mentioned previously, *Campylobacter* spp. only reproduce within an animal's gastro-intestinal tract. Thus it follows that the organism only survives in food or the wider environment; i.e. the numbers of bacteria never increase on an object contaminated with *Campylobacter* spp. In addition, the bacteria are particularly prone to desiccation (Bell, 2006), thus arid conditions (hot or cold) have a detrimental effect on numbers of the bacterium in the environment. This is particularly important for the issue of flock carry-over, where *Campylobacter* spp.

from a previous flock potentially infects the subsequent (previously negative) flock. The short turn-around times between crops, combined with cleaning methods involving a high water usage that thus provides high-humidity conditions required for *Campylobacter* spp persistence. Survival increases the likelihood of flock carry-over. Nevertheless, some doubts remain as to the importance of flock carry-over as a major factor of *Campylobacter* spp. infection in broiler flocks (EFSA, 2004). Whilst some studies have reported the issue to be important (Petersen and Wedderkopp, 2001) others have found no evidence to support this, despite highlighting it as a possibility (Berndtson et al., 1996b; Evans & Sayers, 2000). Perhaps the *Campylobacter* spp. present in a broiler shed after clear-out and cleaning are in a viable but non-culturable (VBNC) and therefore aren't detected via the traditional culturing methods used in such studies. This could be validated via utilizing PCR-based detection methods, as the genetic-based technique is not dependent on the ability of the bacteria to grow on selective agar media. Whilst some authors have reported *Campylobacter* spp. to have a high genetic instability leading to problems tracking the bacteria using these methods (Wassenaar et al., 1998), more recent studies (Klein et al., 2007) were able to use genetic analysis to successfully track strains from farm through slaughterhouse and through to retail meat. This would therefore indicate that the same techniques could be used for investigation into flock carry-over and to determine whether the same source of external infection was responsible for multiple flock colonization. The downside to PCR techniques is that they cannot distinguish between live, dead and VBNC bacteria, and thus there is the risk of isolating genetic material from dead strains rather than VBNC strains (Corry and Atabay, 2001). Nevertheless, even the isolation of DNA from dead strains would possibly still indicate post-clearance sanitation measures were not being carried out thoroughly enough, as these measures should degrade the DNA to a level where PCR would not be able to detect them.

Similarly, PCR methods could be used to analyse the effectiveness of biosecurity measures, attempting to track different strains of *Campylobacter* spp. in and around a broiler farm over the production period, as done by Allen et al. (2008c) to identify strain-association at different farms due to thinning by the same catching teams and/or vehicles (Allen et al., 2008c). Genetic techniques can therefore illustrate how

important the correct hygiene control measures on personnel, vehicles and broiler transport crates are for robust biosecurity standards.

The benefits of improved biosecurity have been demonstrated in terms of reducing the prevalence and spread of infections such as Avian Influenza and *Salmonella* spp.. However, the prevalence of *Campylobacter* spp. in UK poultry meat (FSA, 2009) and the incidence in human *Campylobacter* infections have remained high or even increased (HPA, 2009b) despite the increased biosecurity on farms as a result of avian influenza AI H5:N1 fears since 2004. This indicates that either the measures are insufficient to combat *Campylobacter* spp. propagation in poultry, or that other factors have increasing importance for the incidence of human cases (e.g. a general decrease in behaviour linked to good food hygiene).

Maintaining good biosecurity practices is critical for all aspects of the poultry industry to reduce the likelihood of infection with existing (e.g. *Salmonella* spp. and *Campylobacter* spp.) and emerging (e.g. Avian Influenza) pathogenic microorganisms. With *Campylobacter* spp., the issue is compounded by the low infectious dose needed for the colonisation of a birds' gastro-intestinal tract (as low as 10 wild-type CFU per bird; Newell, 2002). This means that the likelihood of contamination from environmental reservoirs is very high, and can occur from sources such as workers' boots. Although workers and visitors routinely use boot-dips, it is probable that they are not used on every occasion, which could therefore lead to infection of a flock or transfer between infected and uninfected broiler sheds on the same farm. Even when boot dips are used it is plausible that the design of workers' boot treads renders the dipping procedure ineffective. Boots, either steel-toe-capped safety boots or rubber wellington boots almost universally have a deep tread pattern, which can easily carry faecal matter, mud and other material likely to not only harbour *Campylobacter* spp. but protect the bacteria from the effects of boot-dip disinfection. Such material will inevitably become detached, possibly within a broiler shed, leading to contamination of the litter and lead to colonisation of a broiler. As shown by other studies (Evans and Sayers, 2000; Hald et al., 2001; Jacobs-Reitsma et al., 1995), one colonised broiler would lead to rapid infection of the rest of the flock within a short space of time.

For free-range production systems, environmental sources of *Campylobacter* spp. include rivers and streams. However, surface waters surrounding broiler farms are also potentially important reservoirs of the organism. Whilst some degree of contamination may arise due to previous flocks, the importance of contamination from the faecal matter of wild birds is yet to be fully established. In addition to introducing wild-type strains of *Campylobacter* spp., wild birds could also act as a source of other poultry diseases such as Avian Influenza. Identifying methods of reducing the likelihood of interaction between wild birds and broiler systems is therefore of importance to the poultry industry.

Rodents and insects also appear to facilitate the transfer of infection. For instance, rodents have been identified as a source of *Salmonella* spp. infections (Henzler and Opitz, 1992; Heres et al., 2003; Meerburg et al., 2006; Meerburg and Kijlstra, 2007), and insects have been shown to be a vector of *Campylobacter* spp. infection (Hald et al., 2007). Indeed, the distribution of human *Campylobacter* spp. infections has been proposed to be related to the growth cycle of one or more insect species and their access to environmental sources of *Campylobacter* spp. (Nichols, 2005). Studies have demonstrated the ability of *Campylobacter* spp. to colonise insects from poultry sources and subsequently transfer this to other poultry (Shane et al., 1985; Hald et al., 2007). This illustrates the challenges faced by broiler producers in achieving strict bio-security conditions. However, whilst Hald et al. (2007) demonstrated that measures to limit the movement of insects into and out of broiler sheds to be effective in reducing the incidence of positive broiler flocks, the measures have not been widely implemented by the UK broiler industry. Plausibly, this could be due to a combination of lack of awareness of the importance of insects as a vector of *Campylobacter* spp., financial effect of the low profitability per bird, and lastly, the lack of legal requirement to reduce the prevalence of *Campylobacter* spp. in broilers.

Numbers of human and broiler infections peak in early and late summer, respectively, which indicates that factors aside from infected poultry meat are important for the infection of *Campylobacterosis* in humans. Activities such as barbequing have been identified as drivers of seasonal infection (Nichols, 2005). It is likely that most cases arising from barbecuing are due to consumption of undercooked meat, although cross-contamination of raw meat with salads and

propagation by flies may also be important vectors. However, the climate has been reported to be an independent variable affecting the *Campylobacter* spp. colonisation rates in poultry and subsequent rates of human infections (Kapperud et al., 1993; EFSA, 2004).

Throughout the EU there are comparable standards in slaughterhouses, combined with similar production methods for poultry meat by large integrated companies, standard living conditions suggested by multi-national breeding companies such as Avigen and Cobb, and broadly similar methods of transportation. Thus the differences in hygiene, relevant to the control of *Campylobacter* spp., between countries and regions should be relatively small. However, flock prevalence rates throughout Europe vary dramatically, from 3% in Finland to more than 90% in the UK (Rasschaert et al., 2007); which could in part be due to differences in climatic conditions. Peak prevalence occurs at the end of summer and beginning of autumn in moist, temperate countries (UK, Belgium, Netherlands and Northern France; Bouwknegt et al., 2004; Jacobs-Reitsma et al., 1994; Refregier-Petton et al., 2001) as opposed to the hot and dry months earlier in the summer, in part probably due to the bacteria's susceptibility to arid conditions (Bell, 2006; Corry and Atabay, 2001).

In a similar manner to the to the questions asked about the equality of sampling and reporting human cases of *Campylobacterosis* across Europe (EFSA, 2004), one perhaps could question whether or not the incidence in poultry reported in certain areas of Europe is a true indication of the actual incidence, or a reflection on limitations in methodology. EFSA recognise there are problems in treating Europe as a single entity; stating "*EFSA used the available data to indicate general trends in the EU. Owing to differences in reporting and monitoring procedures figures are in most cases not directly comparable between Member States*" (EFSA, 2005). Further discussion is therefore needed with stakeholders in the poultry industry across the EU to develop fair and equitable monitoring and control measures for *Campylobacter* spp. in all Member States.

### 6.3 Harvesting and Transport

Catching has similar biosecurity issues faced during the process of thinning, with catchers, transport crates and vehicles all possible vectors for spreading pathogens.

However, the relative importance is perhaps changed slightly, as at flock clearance the issue of contaminating other birds is not relevant; whilst crate hygiene however is important to prevent the introduction of pathogenic bacteria into previously negative birds en route to the processing plant (Allen et al., 2008a and 2008b; Berrang et al., 2004). Though important, the issue of vehicle and personnel hygiene is not critical for birds on the farm being cleared, but is so to prevent transfer to other farms (particularly at thinning) via the catching teams and vehicles (Corry and Atabay, 2001).

Whilst catching is an important area for biosecurity measures, it is also a critical process from a welfare perspective. As discussed in the literature review of this thesis, manual-catching methods can be a source of physical damage (Scott, 1993) and have a detrimental effect on a bird's wellbeing (Delezie et al., 2007). Manual catching therefore needs to be carefully handled in order to minimise damage to birds (Kettlewell and Turner, 1985) such as bruising and breakages to a bird's breasts, legs and wings. However, the physical nature of the job, combined with the unpleasant working conditions and unsociable hours mean that the workers employed for catching are unlikely to of the type that particularly care about animal welfare. This therefore is constantly working against the goal of high levels of animal welfare, which is further exacerbated by the time pressures often placed upon catchers (De Koning et al., 1987). Taking all of this into consideration, there would seem to be a strong incentive to mechanise the process. Although both methods have been shown to cause some stress to birds, mechanical methods would seem to have less stress than manual methods and birds return to normal more quickly (Delezie et al., 2007). The public perception has been reported to be contrary to this however (Delezie et al., 2007), and whilst a public relations operation could be conducted it is probable that it would be very difficult to "sell" the idea that machines are better than manual catching to the general public, and run the risk that the highlighting of welfare issues to the public would create negative media attention in itself and thus damage the public's perception of the poultry industry.

One of the issues limiting the widespread adoption of mechanical harvesting is house design, as older houses typically have roof support posts that limit the manoeuvrability of catching machines and therefore decrease efficiency. However,

as new houses are built and older ones taken out of use, this issue will be addressed over time. Similarly, issues surrounding purchasing cost and mechanical reliability are likely to become less important over time with increased adoption and further technical development. Because machines do not suffer from fatigue like manual catchers, the rate of catching is more consistent and crucially they do not become less gentle with broilers as fatigue sets in (Delezie et al., 2007). The likelihood is therefore that mechanical harvesting will increase in its usage in the mid- to long-term. It would seem that this will benefit broiler welfare, due to the reported decreases in stress (Duncan et al., 1986) and damage (Knierim and Gocke, 2003), which would have the added benefit of generating less damaged wings and legs during processing.

Once broilers have been loaded into modular crates they are then loaded onto wagon for transportation to the processing factory. Welfare issues predominantly concern the length of transport time together with transportation conditions, particularly heat and ventilation. As shown by the longitudinal survey in Chapter 5, the incidence of wing damage is minimal during transportation. However, biosecurity remains a particularly important issue during transportation, particularly the issue of cross-contamination between different crates within a module. This is exacerbated by crate design, as module crates (drawers) are typically slatted, which permits faecal matter to drop from upper levels onto birds in lower ones. This has been shown to increase both the prevalence and level of pathogens (e.g. *Campylobacter* spp.) on both live birds and subsequently processed carcases (Stern et al., 1995). The solution to this would be solid crate floors to prevent high-risk faecal matter from contaminating lower crates. However, this conflicts with welfare issues, particularly ventilation, which requires the slatted floors to ensure adequate levels of ventilation during transportation and lairage in order to prevent heat-stress and smothering. A radical re-design of the modular transportation systems currently used may therefore be beneficial in order to satisfy both welfare and microbiological requirements.

## 6.4 Primary Processing

Once birds have been transported to the abattoir, they are ideally processed as quickly as possible, though some lairage time is almost inevitable to ensure the constant supply of broilers to the production line. Processing commences with

shackling, where birds are inverted and suspended by their legs on shackles running on a conveyor. There are a number of welfare issues in this process, not only because the birds find human contact stressful, but also because there is the possibility of damage to live birds via vigorous flapping, leg stresses and from contact with factory equipment such as support pillars. Obviously the correct factory design can alleviate the latter issue by removing all hard objects from the vicinity of the suspended live broilers and thus eliminating the possibility of wing damage occurring in this manner.

Similar to catching, shackling welfare can be compromised by the workers who handle the broilers during the process. Rough and improper handling can cause undue stress to the birds and physical damage such as over stressing the leg joints and induce wing flapping, which conversely can be minimised by gentle handling methods during shackling (RSPCA, 2008). Excessive wing flapping can cause wing damage in either of two ways, either by making contact with hard objects as mentioned previously (Jones and Slatterly, 1997), or by being so vigorous that the joints dislocate just by the flapping action. It would seem that this latter issue is addressed by the conclusions of Chapter 6, i.e. that lighter birds, particularly those grown under Freedom Foods standards, are less susceptible to this form of damage, plausibly because they have stronger joints.

Similarly, wing damage caused during carcass processing, namely during de-feathering, can be reduced significantly by these changes to the methods and standards used in production. The findings of the Chapter 6 are significant as they lend credence to the improved welfare standards employed in the RSPCA's Freedom Foods scheme (RSPCA, 2008). Any wing damage in live birds, particularly bone breakage, is likely to cause considerable pain to the bird (Knowles and Broom, 1990). Therefore the incidence of pre-mortem damage, close to zero for Freedom Foods birds, demonstrates how the rearing methodology can be modified to improve welfare standards in live birds.

Chicken wing damage in terms of skin puncturing by wing bones has been shown to occur almost exclusively during the de-feathering process (discussed Chapter 5). This is important as it limits the opportunities for pathogenic bacteria to pass through

the hole in the skin and contaminate the underlying meat. This can therefore not happen during transport or scalding, only during de-feathering and further processing steps such as evisceration. The possibilities of this latter contamination are very limited. Surface contamination of both damaged and undamaged wings should be exactly the same, which therefore accounts for the lack of statistical significance between the three different categories of wing.

Interestingly, de-feathering has been identified as a critical area for the spread of *Campylobacter* spp., as feathers that may be faecally-contaminated are removed and the application of water may promote the transfer of *Campylobacter* spp. from the feathers onto the carcass surface. Although not tested in Chapter 6, this could be a further avenue of investigation. However, when designing the experimental methods, *Campylobacter* spp. were not included due to their high prevalence on normal chicken wings. Initial trials showed that the subcutaneous meat rarely contains pathogenic bacteria, confirming findings by studies such as Berndtson et al. (1992), and that far greater bacterial concentrations were found in samples containing skin. Combined with the practical difficulties in aseptically removal of underlying tissue, it was therefore decided to include skin as part of the sample, in a similar manner to other studies of wing-borne bacterial pathogens (Uyttendaele et al., 1997).

Further on in processing, the evisceration process is critical for a microbiological perspective. The possibility for faecal contamination (e.g. by intestinal rupture) is a major issue for poultry processing as there is potential for a number of human pathogenic bacteria (e.g. *Campylobacter* spp., *Salmonella* spp.) to be transmitted by intestine contents. This is clearly illustrated by the work of Berrang et al. (2004) who found that a statistically significant increase in *Campylobacter* spp. numbers on a carcass could be achieved by the contamination of just 5 mg of ceacal contents. Evisceration therefore is a critical step in the production process and must be managed correctly to minimise the food safety risk to consumers from poultry meat products.

### 6.5 Secondary Processing

It is critical that poultry meets strict standards in terms of food safety, visual appearance and nutritional content. Whilst the latter is a direct result of the nutrition

fed to the bird and therefore solely a production issue, the other two can be undermined within processing (e.g. bruising during shackling), even if things are correctly managed during the rearing phase.

Cross-contamination is a key aspect of this for food safety. There is the potential for raw meat to be contaminated from other raw meat or for workers to contaminate the meat by their actions. Examples of this could be putting cuts of meat back into production after they had dropped onto the floor (instead of disposal) or from a deficiency in personnel personal hygiene or work practices. For instance, it has been discussed how *Campylobacter* spp. can survive on a person's hands for up to 60 minutes (Humphrey et al., 1995). If hand-washing procedures were not followed correctly, this would therefore provide ample opportunity for a worker to transfer infectious doses (can be as low as 500 organisms; Newell, 2002) from raw meat onto RTE (Ready to Eat) products such as cooked chicken breast.

The knowledge assessment surveys conducted on the subject of *Campylobacter* spp. on broiler integrator workers detailed in Chapter 3 are very revealing as although they showed a lack of specific knowledge about *Campylobacter* spp., they did show that the majority of workers had a good grasp of the principles of food hygiene. This is critical for the production of poultry meat with high, consistent standards of food safety. However, worryingly, a limited number of respondents did not show adequate awareness and knowledge of the principles of food hygiene or had a poor attitude towards food safety. Due to the pyramidal nature of the supplier-consumer relationship and the large number of birds typically processed at one site, only a single breach of biosecurity in a processing factory may increase the risks to a large number of consumers. Therefore, in addition to education programs designed to increase awareness of *Campylobacter* spp. issues, some assessment of worker attitudes could be undertaken, linked to their observed behaviours on the factory floor, with a view to determining whether or not a link between the two exists as reported in other studies (Ansari-Lari et al., 2010).

Implementing a form of anti-microbial treatment process for poultry carcasses after evisceration but prior to further processing would seem to both be effective in reducing pathogenic bacterial risk and practical. A number of options have been

discussed in the literature review of this thesis such as various organic acids, namely, lactic, acetic, citric and benzoic acids, (Kim and Marshall, 2000; Hwang and Benchit, 1995; Sakhare et al., 1999; Zeitoun and Debevere, 1990), chemicals such as chorine and hydrogen peroxide (Berrang et al., 2007; Wagenaar and Snijders, 2004) and moist temperature treatments (Avens et al., 2002; De Ledesma et al., 1996; Purnell et al., 2004). Of these, although they show potential benefits, the chemical and organic acid treatments are not currently permitted for use in the EU (Rosenquist, 2008).

This therefore leaves temperature treatments as one of the few options open to processors, which although effective, can give problems with the visual appearance of the retail meat. Freezing birds would seem to be an obvious technique to reduce the risks to consumers, as conducted in some Scandinavian countries to decrease levels of *Campylobacter* spp.. However, consumers in the UK seem to have a preference for fresh poultry as opposed to frozen. The value of fresh poultry sold in the UK is 2.8 times that of frozen (DEFRA, 2009) hence there would implications for the retailing of frozen chicken. Similarly, whilst removal of the skin has been shown to decrease the level of *Campylobacter* spp. on chicken meat (FSA, 2009), some consumers inevitably prefer to buy skin-on chicken.

Chapter 5 of this thesis demonstrates that damaged chicken wings pose no greater to consumers risk in terms of food safety than undamaged wings. Wings have been reported as being a high-risk cut of meat in regards to the presence of *Listeria monocytogens* (Uyttendaele et al., 1997), which was predominantly on the skin rather than in the underlying meat. Therefore this illustrates that the risks to consumers are predominantly from pathogenic bacteria on the skin, particularly when considering that other pathogens such as *Campylobacter* spp. are able to be protected by ridges and folds in the skin (Corry and Atabay, 2001), of which wings have many (certainly *compared* to the stretched skin of breast meat). Feather follicles too have been suggested as a possible area for bacteria to be trapped as a result of the scalding/defeathering process (Berndtson et al., 1992). Both of these are independent of whether a wing is damaged or not, and therefore further illustrate that a wing being damaged does not increase the food safety risk to compared to an undamaged wing.

The evidence gathered in the study may be used in order to change procedures within broiler abattoirs, enabling damaged wings to be graded out later in the production process which gives increased efficiencies due to the utilisation of the full range of machinery. In addition, the work is being used to lobby for a change in legislation to enable damaged wings to be sold. This will not generate large quantities of extra revenue for broiler producers, but it will mean they are able to cut the quantity of waste they have to pay to dispose. With the broiler industry being run under tight profit margins, this could lead to notable benefits in terms of increased efficiency of production (greater yield of meat) and decreased costs.

Similarly, the disposal costs of waste blood have significant implications on the financial performance of many broiler integrators, particularly those located some distance from suitable disposal facilities. The findings detailed in Chapter 4 shows that there is the potential to adequately treat waste blood with types of lime (calcium oxide or hydroxide) which would render the blood safe for spreading onto agricultural land, thereby providing a valuable source of nutrients for the agricultural industry. As mentioned previously, these tests need to be scaled up to a level at which they would be implemented, and the techniques further refined (e.g. how to ensure adequate mixing of the lime and blood). However, if proved to be safe on this level and economical to undertake, then this improvement in resource efficiency would give benefits to both broiler processors and farmers.

### 6.6 Consumers

It has been asserted that with the increases in food safety throughout the food industry, consumers would seem to now be the weakest link in the chain (Brennan et al., 2007). Ultimately, education can only inform people of their choices and of the possible consequences of their actions. There is no legislation that can force people in their own homes to behave in a certain manner to minimise risks to their selves, and thus it follows that the food supplied to consumers has to have high standards of food safety in order to minimise the risk posed thereafter. Within food-borne pathogenic bacteria, *Campylobacter* spp. are a good example of how important consumer behaviour is with regards to the number of human infection as mishandling of poultry meat results in a significant proportion of the total number of *Campylobacterosis* cases in humans (EFSA, 2004; Ghafir et al., 2007; Rosenquist et

al., 2003; Vellinga and Van Loock, 2002). As it is difficult to change people's behaviour, EFSA and the FSA have rather focused on the poultry industry by setting targets to reduce the prevalence of *Campylobacter* spp. in retail poultry, in the hope that this limits the opportunity of human infection even if consumers follow poor food safety practices.

Poultry typically have high levels of *Campylobacter* spp. both on and in them, and whilst the bacterium grows favorably within their gastro-intestinal tract, they are in fact asymptomatic carriers, suffering little adverse effects to the bacteria being present (Newell, 2002). An estimate of 40% of human cases of *Campylobacterosis* has been attributed to poultry products (Ghafir et al., 2007). This leaves the majority of cases of *Campylobacterosis* to come from other sources, such as insects and the environment (Nichols, 2005), together with other foodstuffs including raw milk, (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2004), offal, especially livers (EFSA, 2004; Vellinga and Van Loock, 2002; Whyte et al., 2006).

Interestingly, some human communities in Africa seem to be like poultry – asymptomatic carriers. Newell (2002) states "*Campylobacters can also be recovered from the feces of many asymptomatic adult humans throughout the non-industrialized world, where the infection is considered to be endemic*". Newell suggests that one explanation for this is that the repeated exposure to *Campylobacter* spp. in particularly during infancy, gives a subsequent protective immune response, effective against disease though not always against colonisation.

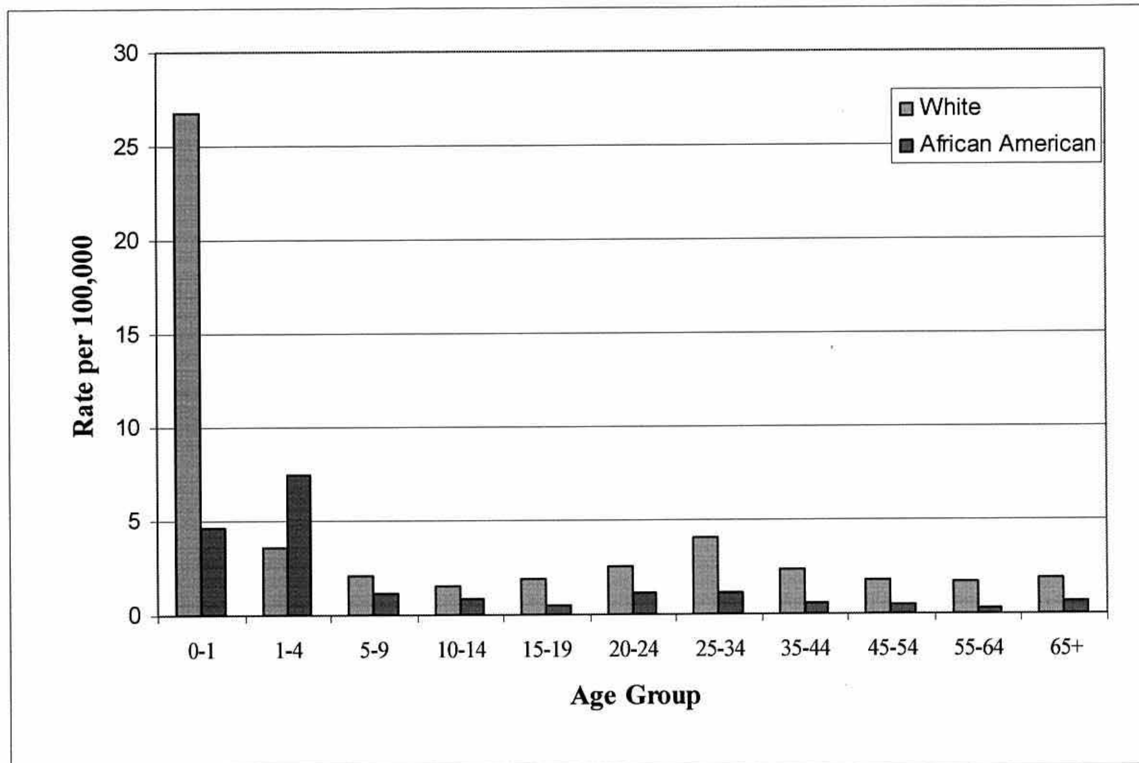


**Figure 6.1** – Recorded cases of *Campylobacterosis* by age and race in the state of Louisiana, USA, 1988-2004. Source data: LDHH, (2007)

The dataset shown in figure 7.1 may indicate a genetic difference in terms of susceptibility to *Campylobacterosis* between different races (White and Afro-American), although it is more probable that these results are more affected by sampling bias. This is because of the nature of the data collection as these figures are from stool cultures, and are only representative of the cases taken to a GP rather than *Campylobacterosis* cases in general. Indeed the LDHH discuss in their 2007 annual report that the high rate of cases in infants are partially due to reporting bias as infants with illnesses are more likely to be taken to medical care and more likely to have stool samples taken than other age groups.

However, an additional influence on this is the access to medical facilities. As private medical facilities predominate in the state (although public funded programs such as Medicaid, CommunityCARE and Kidmed are active) the pattern of lower infection rates in the Afro-American population could potentially be a result of the financial impediment to medical care, rather than simply a genetic difference. This theory has

been confirmed as being highly likely by the head of the LDHH (Rault Raoulard, *Personal communication*, 2008).

Similar influences can occur on any dataset of *Campylobacter* spp. infections (or any other pathogenic infection), and must be borne in mind when reviewing and comparing other datasets from around the world. Indeed the Scottish branch of the FSA has recently published a similar list of factors affecting reporting levels in different areas, and includes immunity through exposure, food consumption, lifestyle and access to medical facilities (FSAS, 2009).

The high levels of infection rates in the 0-1year old category shown in Figure 7.1 are interesting as poultry meat is not thought of as a typical food group for this age group. Therefore, this illustrates the importance of cross-contamination as the cause of these cases, and the effect that a lower level of immunity due to lack of exposure can have, together with the effect of sampling bias.

This curve of infection rates shown in figure 7.1 also supports the theory of some degree of resistance in the general population, even in western countries. However, a spike in the 20-34 year old groups is also observed, particularly in males (data not shown, LDHH, 2007), which is reported to be a result of these social demographics' increased risk-taking and lack of awareness and implementation of good hygiene practices.

### 6.7 Reducing the threat of *Campylobacteriosis* from chicken

A number of issues need to be considered with regards to how best to control *Campylobacter* spp. in poultry and chicken meat. EFSA and the FSA currently are seemingly attempting to eradicate the bacteria from the human food chain, but particularly concentrating on broiler production, in order to cut the number of human cases. If the reduction in levels of *Campylobacter* spp. in broilers was achieved, then initially the number of cases of *Campylobacteriosis* should decrease, and thus a short-term saving in terms of time, money and resources will be achieved. However, Newell (2002) questioned the value of continued endeavours to decrease exposure, stating that the decreased exposure of humans in industrialised countries to *Campylobacter* spp. could lead to increased incidence of the disease as a result of

decreased immunity and lower infectious doses. Immunity against other pathogens has also been reported due to recurring low-level exposure (Silvestro et al., 2004). Indeed, decreasing early childhood infections could increase the infection rates in the adult population as the resistance gained by earlier infections would be removed (Newell, 2002). Additionally, adult cases would 'cost' more to the economy than childhood cases due to loss of working days, productivity etc. Furthermore, it must be remembered that consumption of contaminated chicken meat is estimated to be responsible for only approximately 40% of human *Campylobacterosis* (Ghafir et al., 2007), therefore strategies to reduce infections from other areas also need to be implemented in order that any benefit of a reduction in infections from poultry are not counter-acted by an increase in other areas. Decreasing infections from other areas, such as household pets and the environment, are far more difficult and problematic to implement however.

This developed resistance in humans to pathogenic bacterial colonisation is not confined to *Campylobacter* spp. Indeed, it has been reported (Silvestro et al., 2004) that repeated exposure to other potential pathogenic bacteria, specifically VTEC O157, can produce asymptomatic carriers. Typically *E. coli* O157 is life threatening, and has major complications including renal failure associated with infections. The study's PCR based analysis of stool samples from 400 abattoir and farm workers (who all were without episodes of diarrhoea during the week preceding stool collection), showed that VTEC O157 was isolated from 1.1% of specimens, and further work showed that the wife of one of the positive respondents was also an asymptomatic carrier. Others have hypothesized that immunity to VTEC O157 infection in people living and working on farms due to recurrent exposure to the less virulent and more frequent strains of VTEC non-O157 (Wilson et al., 1996). Although the species of bacteria is not *Campylobacter* spp., it does show the principle behind the theory works in real world situations.

Instead of focusing on removing *Campylobacter* spp. from broiler production, a reduction in the incidence of 'high-risk' broilers (those with high bacterial loads caused by poor pre- or post-slaughter practices) from the food chain should be the goal. Some treatments (chlorination of chill-tanks, carcass dips with organic acid solutions) have been investigated and shown to yield multi-log reductions (Berrang

et al., 2007; Hwang and Benchit, 1995), though chemical decontamination has not been approved for use on broiler carcasses thus far in Europe (Rosenquist, 2008) due to concerns regarding the build-up of bacterial resistance and uncertainty as the effectiveness of certain products (EFSA, 2010). However approved methods such as steam or hot-water dips and sprays and have been shown to be effective (Avens et al., 2002; Whyte et al., 2003), and although visual carcass quality is reported to suffer, this does not affect products which are sold portioned and without the skin on, as the effects of the heat treatments are largely confined to the epidermal layer. Other methods previously discussed are low-heat treatments in the form of freezing (Rosenquist et al., 2006) and the removal of skin from the meat (FSA, 2009). Furthermore, gamma irradiation (Corry and Atabay, 2001) of meat has been reported to be a possibility for decreasing the bacterial load on poultry meat, though doubts remain as to the acceptability by consumers and their practicality in commercial settings. Either alone, or combined with other processing practices employed in the control of *Campylobacter* spp. in other countries, these would decrease the bacterial load upon the carcass and/or processed portions of broiler meat, without necessarily eliminating the bacterium completely. This low level of bacterial challenge would therefore give some of the benefits of continued resistance discussed previously whilst decreasing the risks to consumers.

However, whilst theoretically zero risk to consumers would be ideal, in practice this is not possible; there will always be some level of risk. The FSA report on the incidence of *Campylobacter* spp. in British retail poultry (FSA, 2009) indicates that preventing the bacterium from colonising commercial farms and therefore infecting retail meat is far more difficult than it was envisaged when the target of a 50% cut was created. It is interesting that in the recently published UK government outline strategy Food 2030 (DEFRA, 2010), that the reduction of the incidence of *Campylobacter* spp. is rarely mentioned. Instead *Listeria* spp. are put forward as being the predominant food borne pathogen control, a pathogen of limited significance.

Additionally, the level of risk to consumers should be kept in perspective, as the number of deaths from *Campylobacter* infections remains low. Policies and regulations written for the control of *Campylobacter* need to be achievable to

industry without excessive costs or other implications. Other EU regulations have not considered the ramifications of their implementation, such as the EU Regulation No 1525/98 (Setting Maximum Levels for Certain Contaminants in Foodstuffs such as aflatoxins) where the decrease in risk to consumers was 1.4 deaths per billion (Otsuki et al., 2001). However, the regulation generated a cost of decreased trade exports to the EU of US$670 million (predominantly from Africa) and thus undoubtedly cost many more lives than will have been saved within the EU. Greater research should be conducted into understanding the bacterial/human interaction more fully and the development of treatments such as vaccines.

The extra expense likely to be incurred by producers if further measures are brought in is unlikely to be passed onto consumers. A telling example of consumer behaviour links in with the welfare section of this thesis. Studies have shown a number of consumer contradictions in purchasing behaviour (Harper and Henson, 2001). Firstly, consumers want information on the methods involved in production but wish to avoid connecting the product with the animal, therefore detaching their complicity in its killing. Secondly, in many surveys consumers state they are willing to pay for increased welfare standards, but this rarely translates into purchasing behaviour – cost is still the most important factor at the point of purchase (Harper and Henson, 2001).

Thus, whilst this thesis investigates measures throughout the 'plough-to-plate' production process, from controlling *Campylobacter* spp. in live broilers through to the treatment of poultry blood waste with lime, these are unlikely to be implemented unless the commercial benefits are observed.

### 6.8 Conclusions & Further Work

The experimental chapters of this thesis, as shown in Figure 6.2, focus on a number of areas within bird slaughter and processing, all of which have implications upon consumers and the general public. Chapters such as the microbiology of chicken wings have a direct effect upon both the industry in terms of profitability and consumers in terms of food safety. The study of physical wing damage enhances knowledge surrounding chicken wing damage and therefore has implications for poultry welfare, which is also in the interests of consumers. The study of lime

treatment of waste blood does not directly affect consumers in this way, but does in terms of environmental safety and public health. Finally, the survey of broiler integrator workers on their knowledge of *Campylobacter* spp. and food safety issues encompasses the full plough-to-plate process, highlighting knowledge-gaps that need to be addressed to improve food safety.

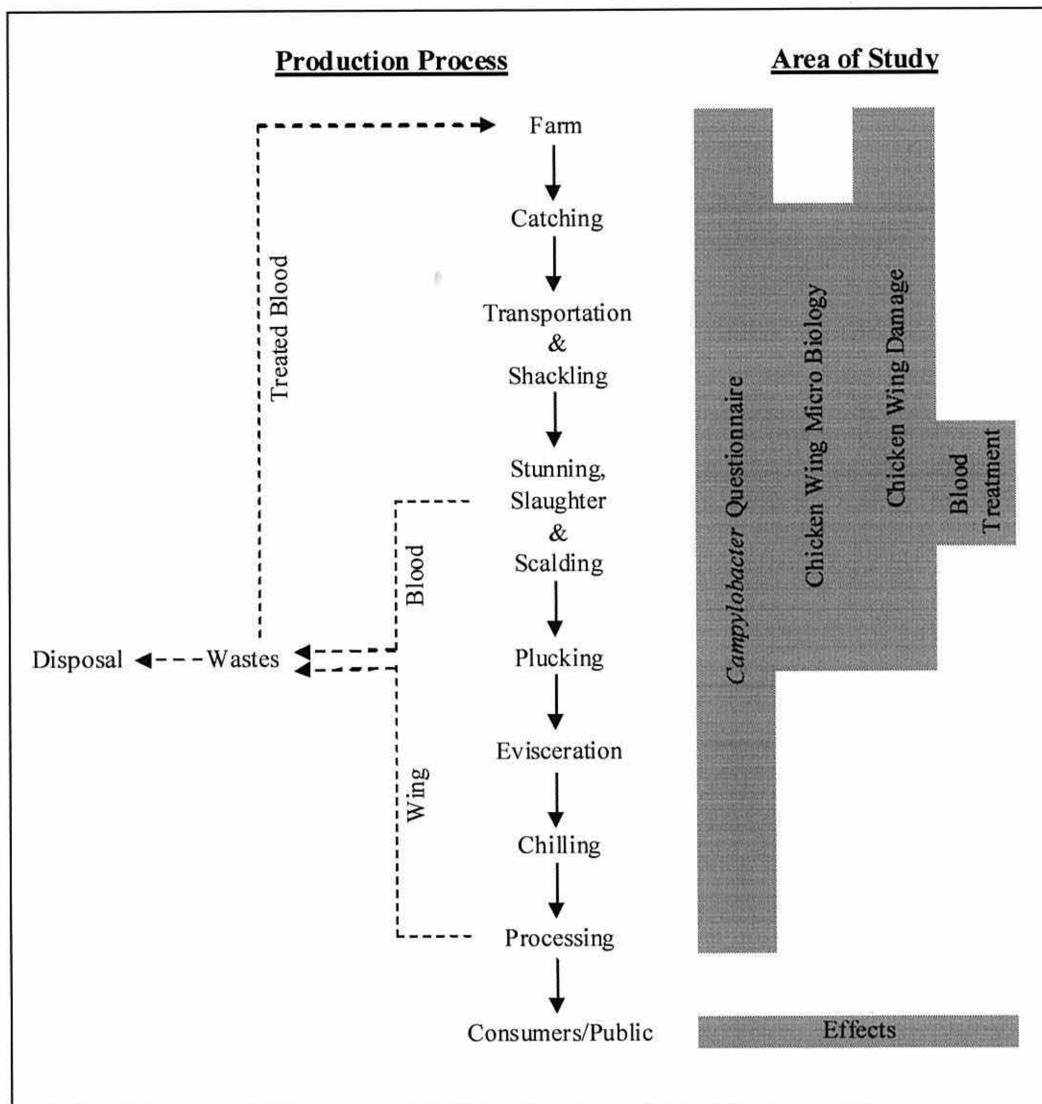


**Figure 6.2** – Production process flow diagram, showing areas covered by research chapters.

## 6.9 *Campylobacter* spp. Questionnaire

Workers from the food industry have been reported to be responsible for food-borne disease outbreaks over many years (Ansari-Lari et al., 2010). Although the survey of broiler industry workers in this thesis showed that general principles of food hygiene practices are widely known, it is still therefore concerning that workers had a lack of specific knowledge surrounding *Campylobacter* spp.. Interestingly, farm workers had more knowledge surrounding *Campylobacter* spp., which is crucial for the successful implementation of preventative measures. However, further educational programs would be of value to reduce the subsequent risks to consumers from factory cross-contamination issues. The findings from the survey could serve as a basis on which to implement an education program for broiler integrator workers. This should be tailored to suit the needs of both farm workers and factory workers, with two different programmes being devised, focusing on areas particularly relevant to their respective job areas. In particular, efforts should be made to highlight to workers the link between their actions and the potential for human infections in order to reinforce the seriousness of the programme.

Furthermore, this could be linked with surveys regarding employee's attitudes and job satisfaction levels, as low levels of the latter have been shown to have a negative effect upon food safety practices (Ko, 2010). A qualitative visual survey of actual practices would also provide further information about the conduct of broiler integrator employees, and would be an interesting addition to the information gathered by the questionnaires.

Finally, there is the possibility to expand the survey to include a number of broiler integrators and processors in order to determine whether the patterns observed in this survey are typical of those in other poultry processing units in the UK.

## 6.10 Wings and Welfare

The chapter on chicken wing microbiology demonstrates that the microbiological health risk posed to consumers from chicken wings is not affected by the wings being damaged. This was further supported by a longitudinal study that found damage in terms of skin-puncturing only occurred during the de-feathering process, crucially after the steps in the production cycle that are most likely to allow ingress

of pathogenic bacteria, i.e. transportation and scalding. This could therefore decrease the amount of waste produced from broiler slaughterhouses, removing the need to treat damaged wings as "not fit for human consumption" and thus lead to increased efficiency in broiler production.

Connected to this, the incidence of chicken wing damage (both pre and post-mortem), was shown to be significantly affected by slaughter weight and by production system used. Notably was the result of just 0.16% for pre-mortem damage in Freedom Foods standards birds ($n$ = 10569). This is important as it provides empirical evidence to support the welfare improvement claims behind Freedom Foods standards broiler production.

To develop and improve on this, the likelihood of increased physical strength in the wings of birds reared under Freedom Foods system compared to conventional systems could be explored in terms of breaking strength. This could be undertaken by obtaining wings of both categories and in turn hold each in a clamp by the humerus and progressively load the lower section of wing (ulna/radius) with increasing weight in order to determine the breaking point of either the bones or the elbow joint. These would have to be undertaken immediately after slaughter in order to negate any effect of the onset of rigormortis and to replicate the situation within the de-feathering machine.

Additionally, further work could be undertaken in order to identify the relative importance of different production factors (breed, enrichment of the rearing environment, nutrition, catching methods and slaughter weight) involved in Freedom Foods production.

Whilst conducting both the physical wing damage research and other unpublished on-farm trials, it was observed that podo-dermatitis levels were very low in RSPCA Freedom Foods birds, and was confirmed by broiler-integrator collected data. It would therefore be interesting to investigate this further, and combine with other welfare indicators such as leg damage, total bruising etc. If a difference was shown to exist, then further studies could identify whether this was due to production factors or the different methodology employed for catching Freedom Foods birds.

## 6.11 Blood Treatment

The lime treatment chapter of this thesis has demonstrated that waste blood from poultry slaughterhouses, even when inoculated with high levels of pathogenic bacteria, can be successfully treated using calcium oxide and calcium hydroxide. Using a bio-luminescent method of analysis in addition to traditional culture methods further reinforces these findings as this is a measure of bacterial activity, not limited by a bacteria's ability to grow in agar plate conditions.

The obvious method for developing this work would be scale up the trials of lime treatment to a scale that would represent the commercial application of the technique. Particular attention should be made to the mixing process in order to ensure that the lime-blood mixture is homogenised fully, leaving no areas that could potentially harbour pathogenic bacteria. Once this is completed, further work identifying the correct application level for both calcium oxide and hydroxide could then be conducted in order to provide data to determine both the technical and financial optimum treatment rate.

If found to be a commercially viable option for poultry blood disposal, this method could give financial savings to poultry producers in addition to providing farmers with a local source of nutrients for applying to their land.

## 6.12 Summary

In summary, the chapters within this thesis have predominantly discussed poultry production microbiology in relation to human safety, be it food safety or environmental safety regarding disposal of poultry waste. This therefore comes down to the issue of risk to humans from the respective bacteria. The issue of risk is sensitive as although one wishes to minimise risk to consumers as far as possible, the minimum level is unlikely to be zero. Indeed, every facet of human life carries some element of risk, from crossing a road to travelling by aeroplane.

Risk management is therefore the key to this, made particularly pertinent by the commercial nature of poultry production, as if the costs involved, for instance in removing *Campylobacter* spp. completely from broiler production, were far in excess of the revenue generated, there would be little point in any broilers being produced.

Realistic risk minimisation can then be undertaken, and by fully understanding the issues faced based on scientific research, the question can be asked as to whether the risks posed justify the money and effort expended.

This is another area in itself however, summed up in 1979, by the then president of the US National Academy of Sciences (P. Handler) who said:

"The estimation of risk is a scientific question... The acceptability of a given level of risk however, is a political question to be determined in the political arena."

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# Appendix

## Example *Campylobacter* Questionnaires and Statistical Tables

## Appendix – Example of *Campylobacter* Questionnaire (English Version)

### Your views about *Campylobacter* risks in Poultry Production

We are collecting views from employees of Vion UK as part of a study to try and improve the safety of chicken meat. You may not have heard of *Campylobacter,* but your views on the bacteria are still important to us. Your views will help us identify areas of knowledge in which we can assist employees.
Any information you provide will be kept confidential and will only be used for the purposes of this research.

It should take under 10 minutes to complete all the questions. Please try to ensure that all questions are answered as this will provide us with a much more detailed and accurate view. Thank you very much for your time.

**How would you describe your job at Vion UK come under:**
*Please tick only one*

Farm worker ☐
Farm manager ☐
Factory worker ☐
Factory manager ☐
Office Staff (at a factory or elsewhere) ☐
Transport employee ☐
Senior Management ☐

**Age** *Please tick*
16-24 ☐
25-34 ☐
35-44 ☐
45-54 ☐
55-64 ☐
65+ ☐

**Gender** *Please tick*

Male ☐
Female ☐

**Education**
*Please tick highest attainment level*
Senior/High school ☐
College ☐
University ☐

**How much have you heard about the following bugs?**
*Please tick one box only on each line*

| | **Nothing** | **A little** | **Quite a bit** | **A lot** |
|---|---|---|---|---|
| *Salmonella* | | | | |
| MRSA | | | | |
| *Cryptosporidium* | | | | |
| *B. subtilis* | | | | |
| Norovirus | | | | |
| *Perginella* | | | | |
| *Clostridium* | | | | |
| *Lactobacillis* | | | | |
| *Listeria* | | | | |
| *Shigella* | | | | |
| *Campylobacter* | | | | |

**How much would you say you know about the bacteria Campylobacter?** *Please circle your personal opinion*

| Nothing | Just the name | A little | Quite a bit | A lot | Expert |
|---|---|---|---|---|---|

**How harmful do you believe Campylobacter is to humans?**
*Please circle your personal opinion*

| None | A little | Somewhat | Very | Deadly | Don't know |
|---|---|---|---|---|---|

**How important is cross-contamination in food safety?**
*Please circle your personal opinion*

| Not at all | A little | some | Quite a bit | A lot | Don't know |
|---|---|---|---|---|---|

**The human symptoms of Campylobacter infection are typically:**
*Please tick any that you believe apply*

| | |
|---|---|
| Watery Diarrhoea | |
| Bloody Diarrhoea | |
| Fever | |
| Vomiting | |
| Stomach Cramps | |
| Head ache | |

**Have you suffered from any of the above symptoms since starting work within the poultry industry?**
Please tick box

| | Prefer not to say | Never | Rarely | Sometimes | Often | Only in the first few weeks |
|---|---|---|---|---|---|---|
| Response | | | | | | |

**If <u>you</u> were to get food poisoning from Campylobacter, what are the most likely ways <u>you</u> would get it?** *Circle only one answer per line)*

| | unlikely | a slight chance | likely | very likely | don't know |
|---|---|---|---|---|---|
| Drinking mains tap water | o | o | o | o | o |
| Handling farm animals | o | o | o | o | o |
| Contact with animal faeces (droppings) | o | o | o | o | o |
| Eating raw vegetables (e.g. salads) | o | o | o | o | o |
| Contact with other people | o | o | o | o | o |
| Streams, rivers ponds & lakes | o | o | o | o | o |
| Contact with household pets | o | o | o | o | o |
| Eating undercooked meat | o | o | o | o | o |
| From soil & mud | o | o | o | o | o |
| Air | o | o | o | o | o |
| Poor kitchen hygiene | o | o | o | o | o |

**How important do you think the following are for the control of pathogens in poultry production, specifically Campylobacter spp.**
*Please circle your personal opinion*

| | None | A little | Some | Quite a bit | A lot | Don't know |
|---|---|---|---|---|---|---|
| Hand Washing | o | o | o | o | o | o |
| Clothing | o | o | o | o | o | o |
| Foot Wear | o | o | o | o | o | o |
| Equipment | o | o | o | o | o | o |
| Chicken Feed | o | o | o | o | o | o |
| Chicken Drinking Water | o | o | o | o | o | o |
| Chicken litter | o | o | o | o | o | o |
| House sanitation after harvest | o | o | o | o | o | o |
| Insect/Rodent Control | o | o | o | o | o | o |
| Air in rearing shed | o | o | o | o | o | o |
| Thinning | o | o | o | o | o | o |
| Catching team | o | o | o | o | o | o |
| Feed withdrawal | o | o | o | o | o | o |
| Transport | o | o | o | o | o | o |
| Stunning | o | o | o | o | o | o |
| Slaughter | o | o | o | o | o | o |
| Plucking | o | o | o | o | o | o |
| Evisceration | o | o | o | o | o | o |
| Chilling (air) | o | o | o | o | o | o |

**In Europe, numbers of Campylobacter are at their peak during the following period?**
*Please tick best response*

Winter ☐
Spring/early summer ☐
Summer ☐
Late summer/autumn ☐
Autumn ☐

**As chickens are transported to a slaughterhouse, do you think:**

| | | | |
|---|---|---|---|
| **Bacterial counts of Campylobacters:** | Increase | Stay roughly constant | Decrease |
| **Number of contaminated Birds:** | Increase | Stay roughly constant | Decrease |

**As chickens travel along the processing line, do you think:**

| | | | |
|---|---|---|---|
| **Bacterial counts of Campylobacters:** | Increase | Stay roughly constant | Decrease |
| **Number of contaminated carcasses:** | Increase | Stay roughly constant | Decrease |

**Thank you very much for you time and thoughts in completing this.**

## Appendix – Example of *Campylobacter* Questionnaire (Welsh Version)

**Eich barn am beryglon *Campylobacter* yn y Diwydiant Dofednod**

Rydym yn casglu barn gweithwyr Vion UK fel rhan o astudiaeth i geisio gwella diogelwch cig cywion ieir. Efallai nad ydych wedi clywed am *Campylobacter,* ond mae eich barn am bacteria yn dal yn bwysig i ni. Bydd eich barn yn ein helpu i ganfod meysydd gwybodaeth lle gallwn helpu gweithwyr.

Byddwn yn cadw'n gyfrinachol unrhyw wybodaeth a rowch, ac ni fyddwn yn ei defnyddio ond at ddibenion yr ymchwil hon.

Dylai gymryd llai na 10 munud i ateb yr holl gwestiynau. Ceisiwch sicrhau eich bod yn ateb yr holl gwestiynau, gan y bydd hyn yn rhoi syniad llawer mwy manwl a chywir inni. Diolch yn fawr iawn am eich amser.

**Ym mha un o'r meysydd canlynol yr ydych yn gweithio gyda Vion UK:**
*Ticiwch y blwch*

*Gweithwyr fferm* ☐
Rheolwyr fferm ☐
Gweithwyr ffatri ☐
Rheolwyr ffatri ☐
Staff Swyddfa (mewn ffatri neu mewn man arall) ☐
Gweithwyr trafnidiaeth ☐
Uwch Reolwyr ☐

**Oed** *Ticiwch y blwch*
16-24 ☐
25-34 ☐
35-44 ☐
45-54 ☐
55-64 ☐
65+ ☐

**Rhyw** *Ticiwch y blwch*

Gwryw ☐
Benyw ☐

**Addysg**
*Ticiwch y lefel uchaf yr ydych wedi ei chyrraedd*
Ysgol Uwchradd ☐
Coleg ☐
Prifysgol ☐

**Faint ydych wedi ei glywed am y bygiau canlynol?**
*Ticiwch un blwch yn unig ar bob llinell*

| | Dim | Ychydig | Eithaf tipyn | Llawer |
|---|---|---|---|---|
| *Salmonella* | | | | |
| MRSA | | | | |
| *Cryptosporidium* | | | | |
| *B. subtilis* | | | | |
| Norovirus | | | | |
| *Perginella* | | | | |
| *Clostridium* | | | | |
| *Lactobacillis* | | | | |
| *Listeria* | | | | |
| *Shigella* | | | | |
| *Campylobacter* | | | | |

**Faint yn eich barn chi ydych yn ei wybod am y bacteria Campylobacter?** *Rhowch gylch o gwmpas eich barn bersonol*

| Dim | Dim ond yr enw | Ychydig | Eithaf tipyn | Llawer | Arbenigwr |
|---|---|---|---|---|---|

**Pa mor niweidiol yw Campylobacter i bobl yn eich barn chi?**
*Rhowch gylch o gwmpas eich barn bersonol*

| Dim | Ychydig | Rhywfaint | Hynod | Yn gallu lladd | Ddim yn gwybod |
|---|---|---|---|---|---|

**Pa mor bwysig yw croesheintio (*crosscontamination*) ym maes diogelwch bwyd?**
*Rhowch gylch o gwmpas eich barn bersonol*

| Dim | Ychydig | Rhywfaint | Eithaf tipyn | Llawer | Ddim yn gwybod |
|---|---|---|---|---|---|

**Fel rheol mae symptomau haint Campylobacter mewn pobl fel a ganlyn:**
*Ticiwch bob bocs sy'n berthnasol yn eich barn chi*

| | |
|---|---|
| Dolur rhydd dyfrllyd | |
| Dolur rhydd gwaedlyd | |
| Gwres uchel | |
| Chwydu | |
| Crampiau stumog | |
| Cur pen | |

**Ydych chi wedi dioddef o unrhyw un o'r symptomau hyn ers dechrau gweithio yn y diwydiant dofednod?**
Ticiwch y blwch

| | Gwell gen i beidio â dweud | Ddim o gwbl | Anaml | Weithiau | Yn aml | Dim ond yn yr ychydig wythnosau cyntaf |
|---|---|---|---|---|---|---|
| Ymateb | | | | | | |

**Pe baech yn cael gwenwyn bwyd o Campylobacter, beth yw'r ffyrdd mwyaf tebygol i chi ei ddal?** *Rhowch gylch o gwmpas un ateb yn unig fesul llinell*

| | Annhebygol | Ychydig o siawns | tebygol | tebygol iawn | Ddim yn gwybod |
|---|---|---|---|---|---|
| Yfed dŵr tap o'r prif gyflenwad | o | o | o | o | o |
| Trin anifeiliaid fferm | o | o | o | o | o |
| Dod i gyswllt â baw anifeiliaid | o | o | o | o | o |
| Bwyta llysiau amrwd (e.e. salad) | o | o | o | o | o |
| Cyswllt gyda phobl eraill | o | o | o | o | o |
| Nentydd, afonydd, pyllau a llynnoedd | o | o | o | o | o |
| Dod i gyswllt ag anifeiliaid anwes gartref | o | o | o | o | o |
| Bwyta cig heb ei goginio'n iawn | o | o | o | o | o |
| O bridd a mwd | o | o | o | o | o |
| Yr aer | o | o | o | o | o |
| Diffyg glanweithdra yn y gegin | o | o | o | o | o |

**Pa mor bwysig yn eich barn chi yw'r canlynol er mwyn rheoli pathogenau yn y diwydiant dofednod, yn benodol Campylobacter spp.**
*Rhowch gylch o gwmpas eich barn bersonol*

| | Dim | Ychydig | Rhywfaint | Eithaf tipyn | Llawer | Ddim yn gwybod |
|---|---|---|---|---|---|---|
| Golchi Dwylo | o | o | o | o | o | o |
| Dillad | o | o | o | o | o | o |
| Esgidiau | o | o | o | o | o | o |
| Offer | o | o | o | o | o | o |
| Porthiant Ieir | o | o | o | o | o | o |
| Dŵr Yfed Ieir | o | o | o | o | o | o |
| Gwellt a charthion ieir | o | o | o | o | o | o |
| Glanhau'r sied ar ôl cynaeafu'r ieir | o | o | o | o | o | o |
| Rheoli pryfed/llygod | o | o | o | o | o | o |
| Aer yn y sied fagu | o | o | o | o | o | o |
| Didoli | o | o | o | o | o | o |
| Tîm dal | o | o | o | o | o | o |
| Tynnu porthiant | o | o | o | o | o | o |
| Cludiant | o | o | o | o | o | o |
| Llonyddu | o | o | o | o | o | o |
| Lladd | o | o | o | o | o | o |
| Pluo | o | o | o | o | o | o |
| Diberfeddu | o | o | o | o | o | o |
| Oeri (aer) | o | o | o | o | o | o |

**Ym mha gyfnod y mae niferoedd Campylobacter yn eu hanterth yn Ewrop?** *Ticiwch yr ymateb gorau*

| | |
|---|---|
| Gaeaf | ☐ |
| Gwanwyn/dechrau'r haf | ☐ |
| Haf | ☐ |
| Diwedd yr haf/hydref | ☐ |
| Hydref | ☐ |

**Wrth i ieir gael eu cludo i ladd-dy ydych chi'n meddwl bod:**

| | | | |
|---|---|---|---|
| **Niferoedd bacteria Campylobacter:** | Yn cynyddu | Yn aros tua'r un peth | Yn lleihau |
| **Nifer y cyrff wedi eu heintio:** | Yn cynyddu | Yn aros tua'r un peth | Yn lleihau |

**Wrth i'r cywion ieir fynd ar hyd llinell brosesu, ydych chi'n meddwl bod:**

| | | | |
|---|---|---|---|
| **Niferoedd bacteria Campylobacter:** | Yn cynyddu | Yn aros tua'r un peth | Yn lleihau |
| **Nifer y cyrff wedi eu heintio:** | Yn cynyddu | Yn aros tua'r un peth | Yn lleihau |

**Diolch yn fawr i chi am roi amser a meddwl i lenwi'r holiadur hwn.**

# Appendix – Statistical Tables

## Chapter 2 - Knowledge gap assessment amongst UK broiler industry workers of the food-borne pathogen *Campylobacter*.

### Employment

**Descriptives**

Score

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Farm | 12 | 16.9583 | 4.21824 | 1.2177 | 14.2782 | 19.6385 | 6.50 | 23.00 |
| Factory | 44 | 10.8409 | 5.92469 | .8932 | 9.0396 | 12.6422 | .00 | 21.50 |
| Office | 37 | 13.8378 | 6.00238 | .9868 | 11.8365 | 15.8391 | 1.00 | 23.00 |
| Total | 93 | 12.8226 | 6.09555 | .6321 | 11.5672 | 14.0779 | .00 | 23.00 |

**Test of Homogeneity of Variances**

Score

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 2.109 | 2 | 90 | .127 |

**ANOVA**

Score

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 416.180 | 2 | 208.090 | 6.238 | .003 |
| Within Groups | 3002.143 | 90 | 33.357 | | |
| Total | 3418.323 | 92 | | | |

**Multiple Comparisons**

Dependent Variable: Score
Tukey HSD

| (I) Job | (J) Job | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Farm | Factory | 6.11742(*) | 1.88092 | .005 | 1.6350 | 10.5999 |
| | Office | 3.12050 | 1.91867 | .240 | -1.4519 | 7.6929 |
| Factory | Farm | -6.11742(*) | 1.88092 | .005 | -10.5999 | -1.6350 |
| | Office | -2.99693 | 1.28828 | .057 | -6.0670 | .0732 |
| Office | Farm | -3.12050 | 1.91867 | .240 | -7.6929 | 1.4519 |
| | Factory | 2.99693 | 1.28828 | .057 | -.0732 | 6.0670 |

* The mean difference is significant at the .05 level.

## Age Group

**Descriptives**

Score

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max | Between-Component Variance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | | |
| 16-24 | | 25 | 8.380 | 6.14797 | 1.230 | 5.8422 | 10.9178 | .00 | 23.00 | |
| 25-34 | | 17 | 14.382 | 5.88305 | 1.427 | 11.3576 | 17.4071 | 2.50 | 22.50 | |
| 35-44 | | 18 | 13.583 | 4.66290 | 1.099 | 11.2645 | 15.9021 | 1.00 | 19.00 | |
| 45-54 | | 22 | 14.977 | 5.73518 | 1.223 | 12.4344 | 17.5201 | 1.50 | 23.00 | |
| 55-64 | | 11 | 14.955 | 4.46909 | 1.348 | 11.9522 | 17.9569 | 7.00 | 21.50 | |
| Total | | 93 | 12.823 | 6.09555 | .632 | 11.5672 | 14.0779 | .00 | 23.00 | |
| Model | Fixed Effects | | | 5.56061 | .577 | 11.6767 | 13.9685 | | | |
| | Random Effects | | | | 1.415 | 8.8934 | 16.7518 | | | 7.83853 |

**Test of Homogeneity of Variances**

Score

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.435 | 4 | 88 | .229 |

**ANOVA**

Score

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 697.327 | 4 | 174.332 | 5.638 | .000 |
| Within Groups | 2720.996 | 88 | 30.920 | | |
| Total | 3418.323 | 92 | | | |

**Multiple Comparisons**

Dependent Variable: Score
Tukey HSD

| (I) AgeGrp | (J) AgeGrp | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 16-24 | 25-34 | -6.00235(*) | 1.74805 | .008 | -10.8709 | -1.1338 |
| | 35-44 | -5.20333(*) | 1.71890 | .026 | -9.9907 | -.4159 |
| | 45-54 | -6.59727(*) | 1.62551 | .001 | -11.1246 | -2.0700 |
| | 55-64 | -6.57455(*) | 2.01191 | .013 | -12.1780 | -.9711 |
| 25-34 | 16-24 | 6.00235(*) | 1.74805 | .008 | 1.1338 | 10.8709 |
| | 35-44 | .79902 | 1.88060 | .993 | -4.4387 | 6.0368 |
| | 45-54 | -.59492 | 1.79564 | .997 | -5.5960 | 4.4062 |
| | 55-64 | -.57219 | 2.15170 | .999 | -6.5650 | 5.4206 |
| 35-44 | 16-24 | 5.20333(*) | 1.71890 | .026 | .4159 | 9.9907 |
| | 25-34 | -.79902 | 1.88060 | .993 | -6.0368 | 4.4387 |
| | 45-54 | -1.39394 | 1.76728 | .933 | -6.3161 | 3.5282 |
| | 55-64 | -1.37121 | 2.12809 | .967 | -7.2982 | 4.5558 |
| 45-54 | 16-24 | 6.59727(*) | 1.62551 | .001 | 2.0700 | 11.1246 |
| | 25-34 | .59492 | 1.79564 | .997 | -4.4062 | 5.5960 |
| | 35-44 | 1.39394 | 1.76728 | .933 | -3.5282 | 6.3161 |
| | 55-64 | .02273 | 2.05339 | 1.000 | -5.6963 | 5.7417 |
| 55-64 | 16-24 | 6.57455(*) | 2.01191 | .013 | .9711 | 12.1780 |
| | 25-34 | .57219 | 2.15170 | .999 | -5.4206 | 6.5650 |
| | 35-44 | 1.37121 | 2.12809 | .967 | -4.5558 | 7.2982 |
| | 45-54 | -.02273 | 2.05339 | 1.000 | -5.7417 | 5.6963 |

* The mean difference is significant at the .05 level.

# Gender

**Group Statistics**

| | Gender | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Score | Male | 51 | 12.7255 | 6.17277 | .86436 |
| | Female | 37 | 13.1351 | 6.24127 | 1.02606 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| Score | Equal variances assumed | .039 | .844 | -.306 | 86 | .760 | -.40964 | 1.33923 | -3.072 | 2.253 |
| | Equal variances not assumed | | | -.305 | 77.224 | .761 | -.40964 | 1.34161 | -3.081 | 2.262 |

# Education

**Descriptives**

Score

| | | N | Mean | Std. Dev | Std. Error | 95% Confidence Interval for Mean | | Min | Max | Between-Component Variance |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | | |
| Highschool | | 41 | 13.305 | 6.3017 | .9842 | 11.316 | 15.294 | .00 | 23.00 | |
| College | | 26 | 11.423 | 5.8952 | 1.1562 | 9.0419 | 13.804 | 2.00 | 21.50 | |
| University | | 19 | 13.579 | 5.5384 | 1.2706 | 10.910 | 16.248 | 2.50 | 23.00 | |
| Unanswered | | 7 | 13.143 | 7.4929 | 2.8320 | 6.213 | 20.073 | 3.00 | 22.50 | |
| Total | | 93 | 12.823 | 6.0956 | .6321 | 11.567 | 14.078 | .00 | 23.00 | |
| Model | Fixed Effects | | | 6.1318 | .6358 | 11.559 | 14.086 | | | |
| | Random Effects | | | | .6358 (a) | 10.799 | 14.846 | | | -.64425 |

**Test of Homogeneity of Variances**

Score

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .737 | 3 | 89 | .533 |

**ANOVA**

Score

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 72.049 | 3 | 24.016 | .639 | .592 |
| Within Groups | 3346.274 | 89 | 37.599 | | |
| Total | 3418.323 | 92 | | | |

**Multiple Comparisons**

Dependent Variable: Score
Tukey HSD

| (I) Education | (J) Education | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Highschool | College | 1.88180 | 1.53725 | .613 | -2.1431 | 5.9067 |
| | University | -.27407 | 1.70174 | .999 | -4.7296 | 4.1815 |
| | Unanswered | .16202 | 2.50764 | 1.000 | -6.4036 | 6.7276 |
| College | Highschool | -1.88180 | 1.53725 | .613 | -5.9067 | 2.1431 |
| | University | -2.15587 | 1.85067 | .650 | -7.0014 | 2.6896 |
| | Unanswered | -1.71978 | 2.61100 | .912 | -8.5560 | 5.1165 |
| University | Highschool | .27407 | 1.70174 | .999 | -4.1815 | 4.7296 |
| | College | 2.15587 | 1.85067 | .650 | -2.6896 | 7.0014 |
| | Unanswered | .43609 | 2.71111 | .999 | -6.6622 | 7.5344 |
| Unanswered | Highschool | -.16202 | 2.50764 | 1.000 | -6.7276 | 6.4036 |
| | College | 1.71978 | 2.61100 | .912 | -5.1165 | 8.5560 |
| | University | -.43609 | 2.71111 | .999 | -7.5344 | 6.6622 |

**Chapter 3 - Lime treatment as an effective sanitization method for *Salmonella* spp. and *E. coli* O157:H7 in abattoir blood waste**

Initial Blood Waste pH

| | |
|---|---|
| CaO | 7.09 |
| | 6.92 |
| | 7.38 |
| $Ca(OH)_2$ | 6.24 |
| | 6.97 |
| | 6.84 |
| $CaCl_2$ | 6.96 |
| | 6.8 |
| Mean | 6.900 |
| SD | 0.322 |
| SE | 0.122 |

**Levene's Test of Equality of Error Variances[a]**

Dependent Variable:pH

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .889 | 11 | 96 | .554 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Chemical + Level + Chemical * Level

**Tests of Between-Subjects Effects**

Dependent Variable:pH

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 934.771[a] | 11 | 84.979 | 1556.799 | .000 |
| Intercept | 10137.162 | 1 | 10137.162 | 185710.458 | .000 |
| Chemical | 535.426 | 2 | 267.713 | 4904.438 | .000 |
| Level | 228.971 | 3 | 76.324 | 1398.230 | .000 |
| Chemical * Level | 170.374 | 6 | 28.396 | 520.203 | .000 |
| Error | 5.240 | 96 | .055 | | |
| Total | 11077.173 | 108 | | | |
| Corrected Total | 940.011 | 107 | | | |

a. R Squared = .994 (Adjusted R Squared = .994)

**Multiple Comparisons**

pH

Tukey HSD

| (I) Chemical Type | (J) Chemical Type | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| CaO | Ca(OH)2 | -.0231 | .05507 | .907 | -.1542 | .1079 |
| | CaCl | 4.7117* | .05507 | .000 | 4.5806 | 4.8428 |
| Ca(OH)2 | CaO | .0231 | .05507 | .907 | -.1079 | .1542 |
| | CaCl | 4.7348* | .05507 | .000 | 4.6037 | 4.8659 |
| CaCl | CaO | -4.7117* | .05507 | .000 | -4.8428 | -4.5806 |
| | Ca(OH)2 | -4.7348* | .05507 | .000 | -4.8659 | -4.6037 |

Based on observed means.

The error term is Mean Square(Error) = .055.

*. The mean difference is significant at the .05 level.

**Multiple Comparisons**

pH

Tukey HSD

| (I) Chemical % | (J) Chemical % | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 0% | 2.5% | -3.3764* | .06359 | .000 | -3.5426 | -3.2101 |
| | 5% | -3.4799* | .06359 | .000 | -3.6461 | -3.3136 |
| | 7.5% | -3.2094* | .06359 | .000 | -3.3757 | -3.0432 |
| 2.5% | 0% | 3.3764* | .06359 | .000 | 3.2101 | 3.5426 |
| | 5% | -.1035 | .06359 | .368 | -.2698 | .0627 |
| | 7.5% | .1669* | .06359 | .049 | .0007 | .3332 |
| 5% | 0% | 3.4799* | .06359 | .000 | 3.3136 | 3.6461 |
| | 2.5% | .1035 | .06359 | .368 | -.0627 | .2698 |
| | 7.5% | .2704* | .06359 | .000 | .1042 | .4367 |
| 7.5% | 0% | 3.2094* | .06359 | .000 | 3.0432 | 3.3757 |
| | 2.5% | -.1669* | .06359 | .049 | -.3332 | -.0007 |
| | 5% | -.2704* | .06359 | .000 | -.4367 | -.1042 |

Based on observed means.

The error term is Mean Square(Error) = .055.

*. The mean difference is significant at the .05 level.

# Blood Waste – Response to Lime Treatment

**Descriptive Statistics**

Dependent Variable: pH

| Chemical % | Chemical Type | Mean | Std. Deviation | N |
|---|---|---|---|---|
| 0% | CaO | 7.2644 | .10737 | 9 |
| | Ca(OH)2 | 7.1644 | .13238 | 9 |
| | CaCl | 7.0867 | .08921 | 9 |
| | Total | 7.1719 | .12995 | 27 |
| 2.5% | CaO | 12.5494 | .25468 | 9 |
| | Ca(OH)2 | 12.5241 | .20890 | 9 |
| | CaCl | 6.5711 | .37094 | 9 |
| | Total | 10.5482 | 2.87901 | 27 |
| 5% | CaO | 12.8637 | .32692 | 9 |
| | Ca(OH)2 | 12.7356 | .28855 | 9 |
| | CaCl | 6.3559 | .14278 | 9 |
| | Total | 10.6517 | 3.10636 | 27 |
| 7.5% | CaO | 12.3269 | .26743 | 9 |
| | Ca(OH)2 | 12.6730 | .25380 | 9 |
| | CaCl | 6.1441 | .15958 | 9 |
| | Total | 10.3813 | 3.06475 | 27 |
| Total | CaO | 11.2511 | 2.35475 | 36 |
| | Ca(OH)2 | 11.2743 | 2.41761 | 36 |
| | CaCl | 6.5394 | .41215 | 36 |
| | Total | 9.6883 | 2.96398 | 108 |

**Levene's Test of Equality of Error Variances(a)**

Dependent Variable: pH

| F | df1 | df2 | Sig. |
|---|---|---|---|
| .889 | 11 | 96 | .554 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+Level+Chemical+Level * Chemical

**Tests of Between-Subjects Effects**

Dependent Variable: pH

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|
| Corrected Model | 934.771(a) | 11 | 84.979 | 1556.799 | .000 | .994 |
| Intercept | 10137.162 | 1 | 10137.162 | 185710.458 | .000 | .999 |
| Level | 228.971 | 3 | 76.324 | 1398.230 | .000 | .978 |
| Chemical | 535.426 | 2 | 267.713 | 4904.438 | .000 | .990 |
| Level * Chemical | 170.374 | 6 | 28.396 | 520.203 | .000 | .970 |
| Error | 5.240 | 96 | .055 | | | |
| Total | 11077.173 | 108 | | | | |
| Corrected Total | 940.011 | 107 | | | | |

a R Squared = .994 (Adjusted R Squared = .994)

**Multiple Comparisons**

Dependent Variable: pH
Tukey HSD

| (I) Chemical Type | (J) Chemical Type | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| CaO | Ca(OH)2 | -.0231 | .05507 | .907 | -.1542 | .1079 |
| | CaCl | 4.7117(*) | .05507 | .000 | 4.5806 | 4.8428 |
| Ca(OH)2 | CaO | .0231 | .05507 | .907 | -.1079 | .1542 |
| | CaCl | 4.7348(*) | .05507 | .000 | 4.6037 | 4.8659 |
| CaCl | CaO | -4.7117(*) | .05507 | .000 | -4.8428 | -4.5806 |
| | Ca(OH)2 | -4.7348(*) | .05507 | .000 | -4.8659 | -4.6037 |

Based on observed means.
* The mean difference is significant at the .05 level.

**Multiple Comparisons**

Dependent Variable: pH
Tukey HSD

| (I) Chemical % | (J) Chemical % | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 0% | 2.5% | -3.3764(*) | .06359 | .000 | -3.5426 | -3.2101 |
| | 5% | -3.4799(*) | .06359 | .000 | -3.6461 | -3.3136 |
| | 7.5% | -3.2094(*) | .06359 | .000 | -3.3757 | -3.0432 |
| 2.5% | 0% | 3.3764(*) | .06359 | .000 | 3.2101 | 3.5426 |
| | 5% | -.1035 | .06359 | .368 | -.2698 | .0627 |
| | 7.5% | .1669(*) | .06359 | .049 | .0007 | .3332 |
| 5% | 0% | 3.4799(*) | .06359 | .000 | 3.3136 | 3.6461 |
| | 2.5% | .1035 | .06359 | .368 | -.0627 | .2698 |
| | 7.5% | .2704(*) | .06359 | .000 | .1042 | .4367 |
| 7.5% | 0% | 3.2094(*) | .06359 | .000 | 3.0432 | 3.3757 |
| | 2.5% | -.1669(*) | .06359 | .049 | -.3332 | -.0007 |
| | 5% | -.2704(*) | .06359 | .000 | -.4367 | -.1042 |

Based on observed means.
* The mean difference is significant at the .05 level.

TVC
Friedman

**Ranks**

| | Mean Rank |
|---|---|
| CaO | 1.33 |
| CaOH | 1.83 |
| CaCl | 2.83 |

**Test Statistics[a]**

| | |
|---|---|
| N | 12 |
| Chi-Square | 14.000 |
| df | 2 |
| Asymp. Sig. | .001 |

a. Friedman Test

**Multiple Comparisons**

Log CFU

Dunnett t (<control)[a]

| (I) Level | (J) Level | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|---|---|---|---|---|---|
| | | | | | Upper Bound |
| 2.5% | 0% | -1.0067* | .11815 | .000 | -.7503 |
| 5% | 0% | -1.0678* | .11815 | .000 | -.8114 |
| 7.5% | 0% | -1.1389* | .11815 | .000 | -.8825 |

Based on observed means.

The error term is Mean Square(Error) = .063.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

*. The mean difference is significant at the 0.05 level.

**Multiple Comparisons**

Log CFU

Dunnett t (<control)[a]

| (I) Lime Type | (J) Lime Type | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|---|---|---|---|---|---|
| | | | | | Upper Bound |
| CaO | CaCl | -1.2908* | .10232 | .000 | -1.0854 |
| CaOH | CaCl | -1.1175* | .10232 | .000 | -.9120 |

Based on observed means.

The error term is Mean Square(Error) = .063.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

*. The mean difference is significant at the 0.05 level.

## Effect of Lime treated blood on grass growth

**Descriptives**

Biomass

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Control | 4 | .7250 | .09292 | .04646 | .5772 | .8728 | .63 | .85 |
| None | 4 | 2.4250 | 1.01359 | .50680 | .8122 | 4.0378 | 1.38 | 3.80 |
| Low | 4 | 2.9275 | 1.01539 | .50770 | 1.3118 | 4.5432 | 1.88 | 4.28 |
| Medium | 4 | 4.7725 | 1.00115 | .50057 | 3.1795 | 6.3655 | 3.89 | 6.12 |
| High | 4 | 3.2175 | 1.48350 | .74175 | .8569 | 5.5781 | 2.21 | 5.36 |
| Total | 20 | 2.8135 | 1.62086 | .36244 | 2.0549 | 3.5721 | .63 | 6.12 |

**Test of Homogeneity of Variances**

Biomass

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.622 | 4 | 15 | .220 |

**ANOVA**

Biomass

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 34.107 | 4 | 8.527 | 8.090 | .001 |
| Within Groups | 15.810 | 15 | 1.054 | | |
| Total | 49.917 | 19 | | | |

**Multiple Comparisons**

Dependent Variable: Biomass

Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Control | None | -1.70000 | .72595 | .186 | -3.9417 | .5417 |
| | Low | -2.20250 | .72595 | .055 | -4.4442 | .0392 |
| | Medium | -4.04750(*) | .72595 | .000 | -6.2892 | -1.8058 |
| | High | -2.49250(*) | .72595 | .026 | -4.7342 | -.2508 |
| None | Control | 1.70000 | .72595 | .186 | -.5417 | 3.9417 |
| | Low | -.50250 | .72595 | .955 | -2.7442 | 1.7392 |
| | Medium | -2.34750(*) | .72595 | .038 | -4.5892 | -.1058 |
| | High | -.79250 | .72595 | .808 | -3.0342 | 1.4492 |
| Low | Control | 2.20250 | .72595 | .055 | -.0392 | 4.4442 |
| | None | .50250 | .72595 | .955 | -1.7392 | 2.7442 |
| | Medium | -1.84500 | .72595 | .133 | -4.0867 | .3967 |
| | High | -.29000 | .72595 | .994 | -2.5317 | 1.9517 |
| Medium | Control | 4.04750(*) | .72595 | .000 | 1.8058 | 6.2892 |
| | None | 2.34750(*) | .72595 | .038 | .1058 | 4.5892 |
| | Low | 1.84500 | .72595 | .133 | -.3967 | 4.0867 |
| | High | 1.55500 | .72595 | .254 | -.6867 | 3.7967 |
| High | Control | 2.49250(*) | .72595 | .026 | .2508 | 4.7342 |
| | None | .79250 | .72595 | .808 | -1.4492 | 3.0342 |
| | Low | .29000 | .72595 | .994 | -1.9517 | 2.5317 |
| | Medium | -1.55500 | .72595 | .254 | -3.7967 | .6867 |

* The mean difference is significant at the .05 level.

**Test of Homogeneity of Variances**

Shoot

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 5.696 | 4 | 15 | .005 |

**Test Statistics[a,b]**

| | Shoot | BioMass |
|---|---|---|
| Chi-Square | 17.485 | 13.243 |
| df | 4 | 4 |
| Asymp. Sig. | .002 | .010 |

a. Kruskal Wallis Test

b. Grouping Variable: Treatment

**Multiple Comparisons**

Shoot

Dunnett C

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| High + Lime | Medium + Lime | -1.1400 | .53216 | -3.9628 | 1.6828 |
| | Low + Lime | .8450 | .51492 | -1.8864 | 3.5764 |
| | Just Lime | 1.7550 | .48947 | -.8414 | 4.3514 |
| | None | 1.9700 | .49215 | -.6406 | 4.5806 |
| Medium + Lime | High + Lime | 1.1400 | .53216 | -1.6828 | 3.9628 |
| | Low + Lime | 1.9850* | .26324 | .5886 | 3.3814 |
| | Just Lime | 2.8950* | .20912 | 1.7858 | 4.0042 |
| | None | 3.1100* | .21532 | 1.9678 | 4.2522 |
| Low + Lime | High + Lime | -.8450 | .51492 | -3.5764 | 1.8864 |
| | Medium + Lime | -1.9850* | .26324 | -3.3814 | -.5886 |
| | Just Lime | .9100* | .16025 | .0600 | 1.7600 |
| | None | 1.1250* | .16826 | .2325 | 2.0175 |
| Just Lime | High + Lime | -1.7550 | .48947 | -4.3514 | .8414 |
| | Medium + Lime | -2.8950* | .20912 | -4.0042 | -1.7858 |
| | Low + Lime | -.9100* | .16025 | -1.7600 | -.0600 |
| | None | .2150 | .05240 | -.0630 | .4930 |
| None | High + Lime | -1.9700 | .49215 | -4.5806 | .6406 |
| | Medium + Lime | -3.1100* | .21532 | -4.2522 | -1.9678 |
| | Low + Lime | -1.1250* | .16826 | -2.0175 | -.2325 |
| | Just Lime | -.2150 | .05240 | -.4930 | .0630 |

Based on observed means.

The error term is Mean Square(Error) = .249.

*. The mean difference is significant at the .05 level.

**Multiple Comparisons**

Dependent Variable: Shoot
Tukey HSD

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 99% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Control | None | -.21500 | .35302 | .971 | -1.6019 | 1.1719 |
| | Low | -1.12500 | .35302 | .042 | -2.5119 | .2619 |
| | Medium | -3.11000(*) | .35302 | .000 | -4.4969 | -1.7231 |
| | High | -1.97000(*) | .35302 | .000 | -3.3569 | -.5831 |
| None | Control | .21500 | .35302 | .971 | -1.1719 | 1.6019 |
| | Low | -.91000 | .35302 | .125 | -2.2969 | .4769 |
| | Medium | -2.89500(*) | .35302 | .000 | -4.2819 | -1.5081 |
| | High | -1.75500(*) | .35302 | .001 | -3.1419 | -.3681 |
| Low | Control | 1.12500 | .35302 | .042 | -.2619 | 2.5119 |
| | None | .91000 | .35302 | .125 | -.4769 | 2.2969 |
| | Medium | -1.98500(*) | .35302 | .000 | -3.3719 | -.5981 |
| | High | -.84500 | .35302 | .170 | -2.2319 | .5419 |
| Medium | Control | 3.11000(*) | .35302 | .000 | 1.7231 | 4.4969 |
| | None | 2.89500(*) | .35302 | .000 | 1.5081 | 4.2819 |
| | Low | 1.98500(*) | .35302 | .000 | .5981 | 3.3719 |
| | High | 1.14000 | .35302 | .038 | -.2469 | 2.5269 |
| High | Control | 1.97000(*) | .35302 | .000 | .5831 | 3.3569 |
| | None | 1.75500(*) | .35302 | .001 | .3681 | 3.1419 |
| | Low | .84500 | .35302 | .170 | -.5419 | 2.2319 |
| | Medium | -1.14000 | .35302 | .038 | -2.5269 | .2469 |

* The mean difference is significant at the .01 level.

## Chapter 4 – Microbiological quality of chicken wings damaged on the farm or in the processing plant

### Coliforms

**Descriptives**

LogCount

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Normal | 88 | 2.2902 | .70612 | .07527 | 2.1405 | 2.4398 | .00 | 3.66 |
| Machine | 88 | 2.4626 | .77518 | .08263 | 2.2983 | 2.6268 | .00 | 4.30 |
| Farm | 88 | 2.4670 | .72323 | .07710 | 2.3138 | 2.6203 | .00 | 3.90 |
| Total | 264 | 2.4066 | .73726 | .04538 | 2.3172 | 2.4959 | .00 | 4.30 |

**Test of Homogeneity of Variances**

LogCount

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .505 | 2 | 261 | .604 |

**ANOVA**

LogCount

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.790 | 2 | .895 | 1.655 | .193 |
| Within Groups | 141.164 | 261 | .541 | | |
| Total | 142.954 | 263 | | | |

## Enterobacteriaceae

**Descriptives**

LogCount

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Normal | 88 | 2.3939 | .61924 | .06601 | 2.2627 | 2.5251 | .00 | 3.88 |
| Machine | 88 | 2.5439 | .75886 | .08090 | 2.3831 | 2.7047 | .00 | 4.49 |
| Farm | 88 | 2.5853 | .69306 | .07388 | 2.4384 | 2.7321 | 1.43 | 4.38 |
| Total | 264 | 2.5077 | .69500 | .04277 | 2.4235 | 2.5919 | .00 | 4.49 |

**Test of Homogeneity of Variances**

LogCount

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.794 | 2 | 261 | .168 |

**ANOVA**

LogCount

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.784 | 2 | .892 | 1.858 | .158 |
| Within Groups | 125.251 | 261 | .480 | | |
| Total | 127.035 | 263 | | | |

**Multiple Comparisons**

Dependent Variable: LogCount
Tukey HSD

| (I) WingType | (J) WingType | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Normal | Machine | -.14995 | .10443 | .324 | -.3961 | .0962 |
| | Farm | -.19132 | .10443 | .161 | -.4375 | .0548 |
| Machine | Normal | .14995 | .10443 | .324 | -.0962 | .3961 |
| | Farm | -.04137 | .10443 | .917 | -.2875 | .2048 |
| Farm | Normal | .19132 | .10443 | .161 | -.0548 | .4375 |
| | Machine | .04137 | .10443 | .917 | -.2048 | .2875 |

**TVC**

**Descriptives**

LogCount

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Normal | 88 | 3.7460 | .55509 | .05917 | 3.6284 | 3.8636 | 2.13 | 5.45 |
| Machine | 88 | 3.7690 | .53100 | .05660 | 3.6565 | 3.8815 | 2.43 | 5.54 |
| Farm | 88 | 3.7730 | .49893 | .05319 | 3.6673 | 3.8787 | 1.43 | 5.36 |
| Total | 264 | 3.7627 | .52696 | .03243 | 3.6988 | 3.8265 | 1.43 | 5.54 |

**Test of Homogeneity of Variances**

LogCount

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| .598 | 2 | 261 | .551 |

**ANOVA**

LogCount

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .037 | 2 | .019 | .067 | .936 |
| Within Groups | 72.994 | 261 | .280 | | |
| Total | 73.032 | 263 | | | |

## *Pseudomonas* spp.

**Descriptives**

LogCount

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Normal | 88 | 2.5013 | .71769 | .07651 | 2.3492 | 2.6534 | .48 | 4.15 |
| Machine | 88 | 2.4121 | .80340 | .08564 | 2.2419 | 2.5823 | .00 | 4.23 |
| Farm | 88 | 2.5960 | .56053 | .05975 | 2.4772 | 2.7147 | .65 | 4.40 |
| Total | 264 | 2.5031 | .70249 | .04324 | 2.4180 | 2.5882 | .00 | 4.40 |

**Test of Homogeneity of Variances**

LogCount

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 4.858 | 2 | 261 | .008 |

**ANOVA**

LogCount

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1.488 | 2 | .744 | 1.513 | .222 |
| Within Groups | 128.301 | 261 | .492 | | |
| Total | 129.788 | 263 | | | |

**Ranks**

| | WingType | N | Mean Rank |
|---|---|---|---|
| Count | Normal | 88 | 133.03 |
| | Machine | 88 | 127.18 |
| | Farm | 88 | 137.30 |
| | Total | 264 | |

**Test Statistics(a,b)**

| | Count |
|---|---|
| Chi-Square | 1.019 |
| df | 2 |
| Asymp. Sig. | .601 |

a Kruskal Wallis Test
b Grouping Variable: WingType

## *Staphylococcus aureus*

**Descriptives**

LogCount

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Normal | 88 | 1.7502 | .45280 | .04827 | 1.6542 | 1.8461 | .00 | 3.08 |
| Machine | 88 | 1.7647 | .52015 | .05545 | 1.6545 | 1.8749 | .00 | 3.43 |
| Farm | 88 | 1.7861 | .39252 | .04184 | 1.7030 | 1.8693 | .00 | 3.15 |
| Total | 264 | 1.7670 | .45663 | .02810 | 1.7117 | 1.8223 | .00 | 3.43 |

**Test of Homogeneity of Variances**

LogCount

| Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|
| 1.433 | 2 | 261 | .240 |

**ANOVA**

LogCount

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .058 | 2 | .029 | .137 | .872 |
| Within Groups | 54.780 | 261 | .210 | | |
| Total | 54.838 | 263 | | | |

## Chapter 5 – Investigation into the incidence of chicken wing damage across different UK broiler production systems

**Group Statistics**

| | Type | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Farm | Llangefni | 21 | 3.4314 | 1.22245 | .26676 |
| | Sandycroft | 11 | 1.0000 | .35214 | .10617 |
| Machine | Llangefni | 21 | 6.9876 | 2.47412 | .53990 |
| | Sandycroft | 11 | 3.9600 | .98225 | .29616 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference Lower | Upper |
| Farm | Equal variances assumed | 8.079 | .008 | 6.413 | 30 | .000 | 2.43143 | .37912 | 1.65716 | 3.20570 |
| | Equal variances not assumed | | | 8.469 | 25.556 | .000 | 2.43143 | .28711 | 1.84076 | 3.02210 |
| Machine | Equal variances assumed | 3.347 | .077 | 3.877 | 30 | .001 | 3.02762 | .78094 | 1.43273 | 4.62251 |
| | Equal variances not assumed | | | 4.917 | 28.658 | .000 | 3.02762 | .61579 | 1.76753 | 4.28771 |

**Group Statistics**

| | Type | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Machine | Sandycroft | 11 | 3.9600 | .98225 | .29616 |
| | Freedom Foods | 6 | 1.5433 | .32408 | .13230 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference Lower | Upper |
| Machine | Equal variances assumed | 6.173 | .025 | 5.782 | 15 | .000 | 2.41667 | .41796 | 1.52580 | 3.30754 |
| | Equal variances not assumed | | | 7.450 | 13.328 | .000 | 2.41667 | .32437 | 1.71766 | 3.11567 |

**Group Statistics**

| | Type | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Farm | Sandycroft | 11 | 1.0000 | .35214 | .10617 |
| | Freedom Foods | 6 | .1617 | .10458 | .04269 |

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Farm | Equal variances assumed | 4.369 | .054 | 5.622 | 15 | .000 | .83833 | .14910 | .52053 | 1.15614 |
| | Equal variances not assumed | | | 7.326 | 12.825 | .000 | .83833 | .11444 | .59077 | 1.08590 |