FINAL REPORT ON FSA PROJECT FS101038

Investigation of the efficacy, practicality and cost effectiveness of modified atmosphere packaging on *Campylobacter* numbers on raw chicken intended for retail

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SUMMARY

Campylobacteriosis is the most common cause of foodborne illness in the UK and epidemiological investigations indicate that handling and consumption of raw or undercooked poultry meat is a significant risk factor. Packing poultry in a modified atmosphere (MA) with a high oxygen concentration has been suggested as a way to reduce the numbers of *Campylobacter* on poultry meat during storage.

This project aimed to:

* Review the existing literature and the current industry use of MA packing of chickens,
* Define the test conditions to be used in the trials,
* Assess the effects of currently used MA gas mixes on *Campylobacter* and other factors such as colour,
* Carry out full scale testing of a proposed new gas mix (Objective 7), and
* Report the results (Objective 8).

The trials were to be carried out on whole fresh chickens.

The review found that the vast majority of whole birds in the UK are packed in air or in a gas mix of 70% or 80% oxygen with 30% or 20% carbon dioxide. Information from the published literature indicates that gas with a high oxygen concentration increases the rate of decline in *Campylobacter* numbers during storage. One paper specifically identifies a mix with 80% oxygen as being optimal.

Preliminary trials concluded that the effects of packing in MA should be compared with packing in air in a high permeability film. Carcasses packed in MA would be in a low permeability film. Aerobic plate counts would be carried out with an incubation temperature of 30°C.

Gas:meat (G:M) ratios were measured on packaged whole birds and portions obtained from two large poultry processors. Two methods of measuring the G:M ratio were tested. The preferred method involved measuring the volume of the unopened pack, the chicken alone, and the packaging alone, by submersion in water. Most of the whole birds had been packed with a G:M ratio of 0.5:1. This ratio was used in subsequent trials.

Measurements of numbers of *Campylobacters* on breast and back skins on whole birds packed in air or MA and stored at 4°C for 3 days showed numbers to be 0.3 to 0.4 $\log_{10}$ cfu/g higher on the back skins. There was no evidence of a difference in the numbers of *Campylobacter* packed in air or MA. One hundred samples were tested to determine numbers of *Campylobacters* in that trial.

Three further trials were carried out to assess the effects of using MA on the numbers of *Campylobacter* on breast skin samples. In the first trial, 120 birds from three sheds were tested after packing in either air or 80%O_2/20%CO_2 and storing for 3 days at 4°C (Day K+3). The breast skin samples contained so few *Campylobacters*, and some showed no indication at all, that counting was not carried out. Gas composition in the packs was measured at the time of packing and just before they were prepared for microbiological testing. Very large variations in oxygen concentration, from about 80% down to 20%, were found between different MA packs. In the second and third trial, again 120 birds were used in each trial. *Campylobacter* numbers were generally low, less than 2 $\log_{10}$ cfu/g. Neither trial showed statistically significant evidence of a difference in *Campylobacter* numbers on breast skins from birds packed and stored in air or MA. The results were analysed together for both air and MA packed birds to look for a relationship between the numbers of *Campylobacter* and oxygen concentration at Day K+3. This analysis showed only very slight evidence of a relationship. There was no evidence of a relationship when considering the birds packed only in MA.

There are a number of differences between this study and those reported in the literature and these might be the cause of the different conclusions regarding the effects of MA. Naturally contaminated carcasses were used in these trials whereas spiked carcasses were used in almost all of the work reported in the literature. A highly permeable film was used to pack carcasses in air and a low permeability film was used to pack carcasses in MA. This is industry practice. Previous studies have not used different films with different gas mixtures. A gas:meat ratio of 0.5:1 was used in the current trials as this is common industry practice. Published trials do not specify the gas:meat ratio used. The birds were packed on-line at the processing plants of one company. Considerable variation in gas compositions in the packs was found at Day K+3. Previous published trials were carried out under controlled laboratory conditions. The birds were tested for *Campylobacter* at Day K+3. Testing after a longer storage period might have shown an effect of MA but this is unlikely as the numbers of *Campylobacter* were low and would have reduced further. With the low starting levels of *Campylobacter*, the trials would never show very large effects of using MA compared to air. The numbers of *Campylobacter* were low despite using rapid on-farm testing to identify positive flocks and testing in both Summer 2013 and Spring and Summer months in 2014.

Following the trials described above, the work was presented to a meeting of the FSA/Industry Joint Working Group on *Campylobacter* -Transport and Processing Subgroup in January 2015. At the suggestion of the Group, a revised work plan was submitted but after assessment and review the project was terminated by the FSA in May 2015 as it considered continued funding “would not represent value for money/add to the value of the Agency’s *Campylobacter* portfolio”.

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Appendix 1: Library of Literature on MA Effects on Poultry

1. INTRODUCTION

Campylobacter species (including *C.jejuni and C.coli*) are the most common cause of bacterial foodborne disease in the UK and are transmitted most often from poultry (BBSRC/FSA/Defra, 2010). The prevalence of *Campylobacter* on poultry in the UK has been reported as 86% (EFSA, 2010) and more recently as 73% in an FSA-funded retail survey (Jorgensen *et al.*, 2015). Reducing the incidence of human *Campylobacter* infection requires interventions at the farm, at the slaughterhouse, and during subsequent storage, transport, and at retail. The work described in this report addresses the latter stages of post process storage, distribution, and retail.

Generally, poultry plants pack whole birds either by (a) over-wrapping on to a tray and then into a mother bag with a modified atmosphere (MA) mixture for transport to the distribution depot and retail outlet, or (b) over-wrapping onto a tray and put into a crate with no MA, or (c) MA packed. Alternatively, birds are (a) portioned and MA packed or (b) sent to a packing plant where the carcasses are portioned and MA packed. WRAP (2010) describes the relative merits of various pack formats with gas-flushed high-barrier shrink-film offering many advantages in terms of shelf life, pack weight, and packing speed.

A range of gas mixtures can be used. Previous advice from industry has been to use a 70% O_2 + 30% CO_2 mixture for packing skin-off portions and a 30% CO_2 + 70% N_2 mix for packing whole birds (Air Products, 2004). The latter mixture was chosen to restrict fat rancidity. The mixtures were chosen to increase shelf life and were not focussed on reducing *Campylobacter*. In general, CO_2 is used to restrict the growth of aerobic bacteria and increase shelf life (minimum concentration of 20%) but high levels can cause drip loss and odour problems (Air Products, 2004). High concentrations of O_2 can maintain colour and research described in the literature shows that it can reduce *Campylobacter* but fats, that are mostly associated with the skin, may be oxidised. As CO_2 is quite soluble in meat, N_2 is often used as a filler to exclude air and maintain gas pressure in packs (Air Products, 2004). Campylobacters are generally considered not to multiply in aerobic environments or at temperatures below about 32ºC. They survive most in a moist environment at temperatures close to, but above, freezing. *Campylobacter* numbers will fall during the shelf life of poultry.

Clearly, there are competing factors acting during storage and these can result in poultry processors using different gas mixtures, often as requested by the retailer. The overall goal of this project was to quantify the effectiveness of gas mixtures currently used by industry, and to define how and what gas mixtures will induce the most rapid decline in numbers of *Campylobacter* on raw poultry during storage in ways that are practical and cost effective.

2. STRUCTURE OF THE PROJECT

The following table shows the objectives for the project.

OBJECTIVE NUMBER	OBJECTIVE DESCRIPTION
1.	**TO COLLATE AND INTERPRET DATA FROM THE SCIENTIFIC AND TECHNICAL LITERATURE AND FROM INDUSTRY EXPERIENCE.** To identify the reasons for using different gas mixes and their effects (shelf life, colour, drip, Campylobacter reduction).
2.	**TO COMPARE THE EFFECT OF STORAGE IN AIR IN A SEALED PACK WITH STORAGE IN AN OVERWRAPPED TRAY.** To provide evidence on the effect of overwrapping. Should overwrapped birds be the controls for comparison with MAP. **TO COMPARE THE EFFECT ON APC OF INCUBATING PLATES AT 20 or 30°C.** To define the incubation temperature to be used in further tests.
3.	**TO MEASURE THE GAS:MEAT RATIO.** To measure the ratios currently used by industry and define the ratio to be used in further tests.
4.	**TO QUANTIFY THE EFFECT ON MICROBIAL COUNTS, DRIP, COLOUR, RANCIDITY AND ODOUR OF GAS MIXES CURRENTLY USED BY THE POULTRY INDUSTRY.** To identify the best currently used gas mix in terms of Campylobacter reduction whilst noting the effects on other organisms and factors (drip, rancidity, odour, colour).
5.	**TO ASSESS THE VARIATION IN CAMPYLOBACTER COUNTS AROUND MODIFIED ATMOSPHERE PACKED (MAP) CARCASSES (TOP BREAST SKIN VERSUS BACK SKIN).** To identify any variations in the effectiveness of the treatment.
6.	**TO IDENTIFY THE OPTIMUM GAS MIX BASED ON EFFECTS ON MICROBIAL COUNTS, DRIP, COLOUR, RANCIDITY AND ODOUR.** To define the optimum gas mix based, in part, on the results from Objective 4.
7.	**TO CARRY OUT TEMPERATURE ABUSE TESTING, DEFINE PRACTICALITY OF THE PROPOSED GAS MIXTURE, AND CARRY OUT A FULL SCALE TRIAL.** Gas suppliers and packaging companies to define costs. Poultry processors and retailers to provide views on practicality.
8.	**REPORTING, AND INFORMATION ON PRACTICALITY AND COSTS.**

This report describes the activities to meet those objectives. All of the packing was carried out by a poultry processor using in-line production packing equipment.

3. REVIEW OF THE LITERATURE ON THE EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON *CAMPYLOBACTER* AND INFORMATION ON THE GAS MIXES CURRENTLY USED BY INDUSTRY (OBJECTIVE 1)

3.1 Introduction to the Review

There have been two previous reviews discussing the effectiveness of modified atmosphere packaging (MAP) in reducing the numbers of campylobacters on poultry meat at retail (Alter and Scherer, 2006; Farber, 1991). This project aimed to bring up to date these previous studies, by identifying and reviewing the more recent peer-reviewed and technical literature relating to the effect of gas mixtures that are currently used, or have been recently assessed. In addition to the microbiological consequences of MAP, the review considered the effects of different compositions of the gas mixtures on drip, rancidity, colour and odour where data is available. A systematic approach was used to identify relevant peer- reviewed literature to draw conclusions for the survival of *Campylobacter* and selected spoilage bacteria.

The review also aimed to summarise the gas mixtures recommended by gas suppliers and those currently used by poultry processors.

3.2 Currently used gas mixtures

3.2.1 Recommendations from gas suppliers

In 1990, Campden BRI formed a Modified Atmosphere Packaging Club which included representatives from gas and packaging suppliers, the food industry, retail, and the UK Ministry of Agriculture, Fisheries and Food. The club produced a technical manual that provides guidelines on the MA packing of food products (Day, 1992). The recommendations in those guidelines are still included in the literature from gas and equipment suppliers as will be shown below.

The guidelines recommend using a mix consisting of 25-35% CO_2 and 65-75% N_2 for retail packs of skin-on poultry. For bulk packing, the advice is to use 80-100% CO_2 and 0-20% N_2. The CO_2 retards the growth of aerobic bacteria increasing the lag phase and generation time of susceptible spoilage microorganisms. However, CO_2 can increase the growth of some microorganisms, such as lactic acid bacteria. Another problem with using CO_2 is that it is absorbed by water and fat which can lead to pack collapse: this is the reason for the addition of the N_2 in the retail packs. Pack collapse is not an issue for bulk systems. Too much CO_2 can lead to high concentrations of carbonic acid, changes in pH, and excessive drip and this is the reason, along with pack collapse, why the retail packs were restricted to around 30% CO_2. The N_2 acts as a filler gas which has low solubility in both water and fat. It displaces O_2 and so inhibits aerobic spoilage and oxidation.

The guidelines were developed further with the industry and the literature summarised in Table 1 shows the additional guidance on packing of skin-off poultry. In this case, the recommendations are that retail and bulk packs should contain 70% O_2 and 30% CO_2. The CO_2 restricts aerobic spoilage whilst the oxygen maintains the reddish colour for longer periods. With skin-less portions the fat layer has been removed so oxidation is not such a concern, however, the O_2 does reduce the shelf life slightly compared to the use of N_2. The most recent guidance from Air Products (2005) suggests that a mix of 80% O_2 and 20% CO_2 is beneficial in the reduction of *Campylobacter*. As will be seen later from the review of the refereed literature, high O_2 levels are thought to reduce the numbers of *Campylobacter*, whilst the inclusion of 20% CO_2 provides adequate shelf life.

Table 1. Information taken from the Campden BRI Guidelines (Day, 1992) and literature of the Gas and Equipment Suppliers

Source	Product	Temperature °C	Gas Mix, %			Gas:meat ratio	Shelf life in air, days	Shelf life in MAP, days
			O_2	CO_2	N_2			
Day (1992)	Chicken (retail)	-1 to 2		25-35	65-75		4 to 7	10 to 21
	Chicken (bulk)	-1 to 2		80-100	0-20		4 to 7	10 to 21
Air Products (2004)	Chicken skin-on (retail)	-1 to 2		30	70		4 to 7	10 to 21
	Chicken skin-on (bulk)	-1 to 2		100			4 to 7	10 to 21
	Chicken skin-off (retail)	-1 to 2	70	30		2:1	3 to 5	7 to 14
	Chicken skin-off (bulk)	-1 to 2	70	30			3 to 5	7 to 14
Linde Group (2012)	Light poultry	2 to 3		40-100	0-60	1:1 to 2:1	4 to 7	16 to 21
	Dark poultry	2 to 3	70	30		1:1 to 2:1	3 to 5	7 to 14
Linde Group (2013)	Poultry	1.7 to 3.3		50-80	20-50	1:1 to 2:1	7	16-21
Matheson Tri Gas(2009)	Capon chicken, skin-off chicken, sliced dark poultry (retail)		70	30				
	Capon chicken (bulk)			100				
	Skin-off chicken, sliced dark poultry (bulk)		70	30				
PBI Dansensor	Chicken (retail)			30	70			
	Chicken (bulk)			100				
	Skin-off chicken (retail)		70	30				
	Skin-off chicken (bulk)		70	30				

A technologist at Air Products (March 2013) provided further information of their current advice and the gas mixes being used by their customers in the packing of chicken portions or mince:

* 70% O_2 and 30% CO_2 (0% N_2) – This is the mixture that is widely reported, as seen in Table 1. As commented above, it is used to keep the "red" colour of the meat but the shelf life is reduced slightly due to the high O_2 content.
* 20% O_2, 30% CO_2, and 50% N_2 – This mixture is being recommended more and more frequently and has sufficient O_2 to maintain the colour of the poultry and prolongs shelf life longer than the 70% O_2 and 30% CO_2 mix. Also, at this O_2 level which is close to that of air, there is no need for an anti-deflagration pump.
* 50% O_2, 30% CO_2, and 30% N_2 – This mix is sometimes used but the level of CO_2 can cause some packaging to collapse.
* 15% O_2, 70% CO_2, and 15% N_2 – A few processors use this mix and achieve good shelf life. It causes some collapse of the packs but that is not so important for these processors.

3.2.2 Gas mixtures in current use

Table 2 shows gas mixes measured prior to the project in February 2012 by Campden BRI in packs of poultry obtained from the outlets of major UK retailers. Most of the mixes fall into two groups. Eight of the 24 samples had a high O_2 concentration (~80%). Another group had O_2 concentrations around 20 to 30%. One of the packs had a very high CO_2 concentration.

Table 2. Gas mixes measured in packs of raw poultry bought from outlets of major UK retailers (February, 2012) * Pack had leaked; ** Pack had blown; ***Vacuum packed

Retailer	Product	Skin on/off	Measured gas concentration, %		
			O_2	CO_2	N_2
A	Whole bird	On	23	10	67
A	Whole bird	On	20	6	74
A	Whole bird	On	17	8	75
A	Breast	Off	21*	4*	75*
A	Thighs	On	0	16	84
B	Whole bird	On	78	16	6
B	Whole bird	On	69	20	11
B	Breast	Off	79	18	3
B	Drums	On	41	12	47
C	Whole bird	On	23	9	68
C	Whole bird	On	14	20	66
C	Breast	Off	79	19	2
C	Breast	On	81	18	1
C	Breast in separate bags	Off	0	85	15
C	Thigh and drum	On	79	18	3
D	Whole bird	On	19	12	70
D	Whole bird	On	28**	14**	58**
D	Breast	Off	21	1	78
D	Thighs	On	79	17	4
E	Thighs	On	75	18	7
F	Whole bird	On	75	17	8
F	Breast	On	25	20	55
F	Breast	Off	81	16	3
F	Breast in separate bags	Off	***	***	***

In this project, packs have been obtained immediately after packing by two processors and the gas mixes in the packs measured on that day (Table 3). Eight of the ten packs tested contained a gas mix with 80 to 90% O_2. The other packs contained around 25% O_2, 20%CO_2 and 55% N_2. In addition, five major processors and two retailers provided data on the gas mixes that they use (Table 4). Six mixes, not including air, were noted with the vast majority of whole birds, and breast portions, packed in an 80% O_2 and 20% CO_2 mix. The five other mixes used O_2 concentrations of 60% (with 20% CO_2) 40% (with 10% CO_2), 20% (with 30% CO_2), 16% (with 22-25% CO_2) and <3% (with 27 to 35% CO_2).

Table 3. Gas mixes measured in packs of raw poultry obtained directly from the packing lines of two poultry processors

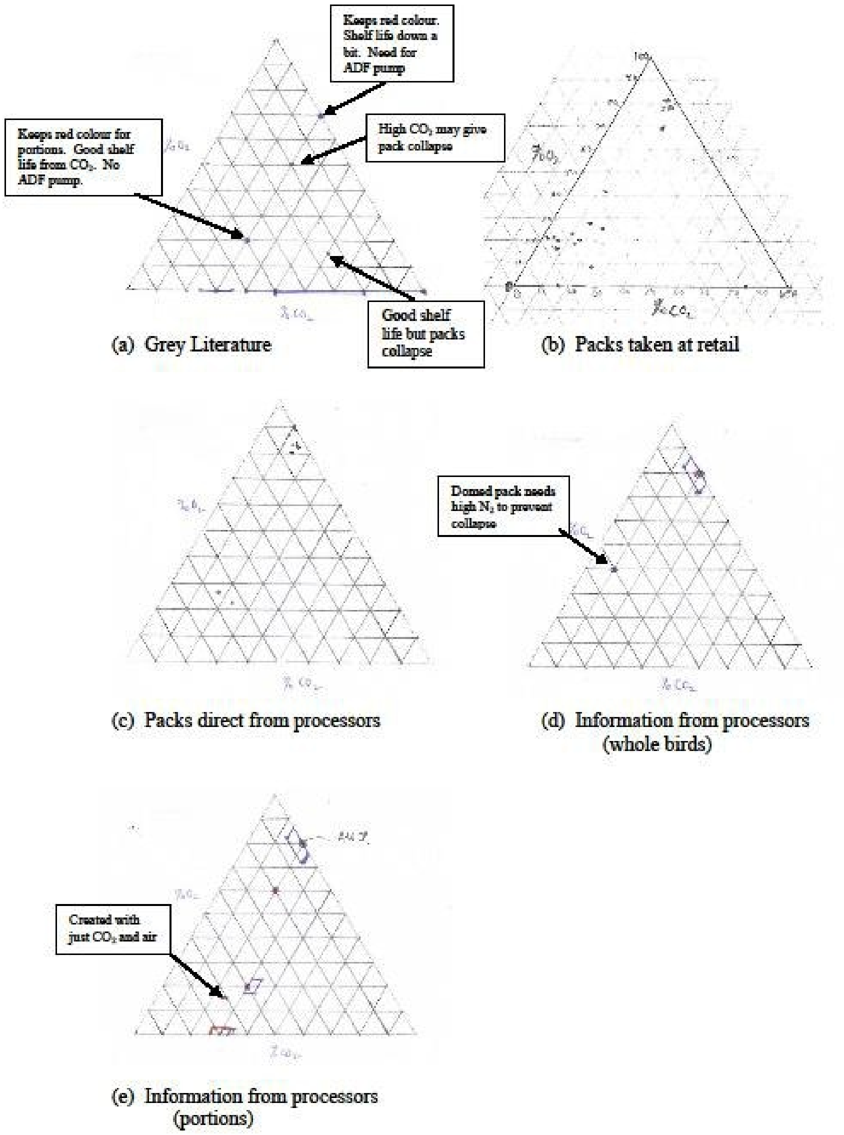
* O_2 and CO_2 concentrations are measured and N_2 calculated as the remaining percentage

Product	Skin on/off	Measured gas concentration, %		
		O_2	CO_2	N_2
Thigh	On	27.2	16.2	56.6
Drum	On	23	24.1	52.9
Breast, mini fillets	Off	84.5	13	2.5
Breast	On	86.9	10.4	2.7
Breast, fillet	Off	89.9	10.7	-0.6*
Whole bird	On	80.3	14.3	5.4
Breast	Off	81.8	16	2.2
Breast, diced	Off	83.6	14.9	1.5
Thigh and drum	On	81.8	16.4	1.8
Drum	On	82.3	14.6	3.1

Table 4. Gas mixes in current use as advised by processors and a retailer

Product	Skin on/off	Measured gas concentration, %		
		O_2	CO_2	N_2
Whole bird	On	80	20	
Whole bird	On	40	10	50
Whole bird	On	Air	Air	Air
Portion	On or off	80	20	
Portion	On	60	20	20
Portion	On	20	30	50
Portion	On	16	22-25	58-62
Portion	Off	0-3	27-35	63-78 (Balance)

The following graphs (triangular co-ordinates) summarise the data obtained from the gas suppliers (grey literature), measurements on packs from retail outlets and processors, and information from the processors. It shows that in the UK, apart from the whole birds packed in air by two processors, all whole birds from the major processors are packed in a mix of 80% O_2 and 20% CO_2 except for one pack type which uses a dome and requires a higher N_2 concentration. Five mixes are used by the major processors to pack portions in the UK. Since carrying out this review, some processors have been found to be using 70% rather than 80% oxygen in the gas mixture.



3.3 A systematic search of the peer-reviewed literature

The objective was to critically review and evaluate the effectiveness of MAP to reduce numbers of campylobacters on chicken meat. The approach adopted was based on the methodology of a systematic review (Jadad *et al*., 2000). The Thompson ISI electronic database, PubMed and Medline were searched with defined search strings. Selection criteria were then used to remove irrelevant references and a secondary library was produced on the basis of title, keywords and abstracts.

The Boolean search strings were:

> *Campylobacter AND Chicken AND [packaging AND (food preservation OR drip OR rancidity OR colour OR odour)]*
> *Campylobacter AND (gas OR oxygen OR carbon dioxide)*
> *Chicken AND Packaging (Pseudomonas OR Enterobacteriaceae)*

The titles and abstracts were screened for relevance using the following criteria:
(1) Any reference not pertaining to the viability of *Campylobacter, Enterobacteriaceae or Pseudomonas* was removed
(2) Any reference not pertaining to *Campylobacter jejuni or C. coli* or other thermophilic campylobacters or unspecified campylobacters were removed.
(3) Any duplicated references were also removed.
A total of 62 references passed screening (Table 5). The final library is provided as Appendix 1.

Table 5. Results of literature search

Source database	Number of references
Medline Ovid	78
Web of Knowledge	452
Other	9
Total references	539
Total after removing duplicates	487
Total after removing non English articles	475
Total after relevance screening	74

3.4 Considerations for Campylobacters when using a MAP

C. coli and *C. jejuni* are thermophilic, highly motile, Gram-negative, spiral forming bacteria (Cowan and Steel, 1993). They are the most common cause of human food borne illness with poultry as a major source (Kudra *et al*., 2012). Generally, *Campylobacter* compete poorly with other bacteria on packaged chicken (Farber, 1991). However there is evidence of survival of *Campylobacter* jejuni for an extended period, greater than 48 hours, in a biofilm that includes Pseudomonas (Bronowski et al., 2014) Campylobacters are also capable of extended survival in the absence of any significant competing microflora (Davis and Conner, 2007). Campylobacters are also particular with regard to their growth requirements and fragile if handled inappropriately. Despite these properties, however, campylobacters can thrive the farming of their chicken hosts, the chicken slaughter process and the retail supply chain. *Campylobacter* lacks various ubiquitous stress response factors, most importantly the oxidative stress response factor and the stationary phase stress response factor which reduce its ability to compete with other micro-organisms. The ability to shift metabolism into a viable but non-culturable state (VBNC) and high genetic diversity within the *Campylobacter* genera are considered to be largely responsible for their survival in unfavourable environments (Alter and Scherer, 2006).

3.4.1 Campylobacters and the effect of temperature on survival

C. jejuni and *C. coli* are thermophilic *Campylobacter* (Park *et al*., 1991) with growth mostly occurring between 37-42°C and not below 30°C. Blankenship and Craven (1982) reported a 1-2 $\log_{10}$ cfu/g increase in *C. jejuni* numbers over 4 days when inoculated ground chicken was stored at 37°C in an ambient atmosphere. In comparison, there was no growth observed when identical preparations were stored at 4°C or 23°C. Similar observations were reported in a later study which also determined there was no increase in *Campylobacter* numbers during the storage of refrigerated packaged (in air) chicken (Jacobs-Reitsma, 2000). Although no growth was observed from these two studies, considerable metabolic activity can be detected in*Campylobacter* cells at temperatures as low as 15°C (Kelly *et al.*, 2003). At temperatures as low as 4°C, campylobacters have been observed to operate metabolic processes in the form of oxygen consumption, catalase activity, ATP generation and protein synthesis (Hazeleger *et al.*, 1998). Kam Fai Chan *et al.* (2001) also reported survival of campylobacters at 4°C when there was substantial genetic variability among the populations being chilled. Furthermore, clinical campylobacters were significantly more likely to be viable following chilled storage compared with poultry-derived strains. Limited survival of campylobacters was also reported after storage at lower temperatures of -20°C (Kam Fai Chan *et al.*, 2001). However, it was suggested that such survival was a consequence of atypical genetics and that consequently only a few genotypes were able to tolerate such extreme conditions. At-20°C, there was no significant difference in survival when clinical or avian sources were compared (Kam Fai Chan *et al.*, 2001).

3.4.2 Campylobacters and the effect of oxygen on survival

The correct reduction-oxidation (redox) potential is vital for *Campylobacter* growth (Park *et al.*, 1991) and the organism multiplies optimally in 5% oxygen (Farber, 1991) when cultured at thermophilic temperatures of between 30°C and 42°C (Park *et al.*, 1991). Two key regulators of oxidative stress defence, *Sox*RS and *Oxy*R define genes, are not present in *C. jejuni*, thus the recognition and response to oxidative stress is not mediated by these classic mechanisms (Parkhill *et al.*, 2000). However, there is some evidence to support alternative oxidative stress mechanisms and *Campylobacter* is not always as fragile as it is often supposed in the laboratory. Jones *et al.* (1993) reported growth of *C. jejuni* after four days of incubation on blood agar in air (after an initial 18h in microaerobic conditions) and continued growth for >3 weeks. This adaption to growth in air was accompanied by a morphological change from a spiral to a coccoidal form and the air-adapted organism grew equally well when subcultured back into a microaerobic atmosphere.

3.4.3 Campylobacters and consideration of motility with regard to survival when using a MAP

Campylobacter is a highly motile organism due to the presence of polar flagella. Hazeleger et al. (1998) reported that *C. jejuni* displayed positive chemotaxis towards formate and malate, and negative aerotaxis in soft agar. The extent of the chemotaxis depended on substrate availability in the agar and was measured by distance moved across the agar. *C. jejuni* was also reported as able to migrate to favourable microaerophilic niches within ground chicken meat when incubated at 37°C in an ambient-atmosphere (Blankenship and Craven, 1982). An ability to move to locations with favourable atmospheres is an important consideration when attempting to reduce *Campylobacter* numbers on chicken by packaging in MAP.

3.5 Bacterial spoilage of poultry meat

Spoilage of food is generally associated with microbial consumption of nutrients such as sugars, free amino acids and the release of undesired volatile metabolites (Ercolini *et al.*, 2009). Numbers of spoilage organisms higher than 10^7 cfu/g or ml of food may result in off flavours, off odours and visual defects (Air Products, 2013) caused mostly by the catabolism of carbohydrates (Russell *et al.*, 1995). Poultry meat shelf-life depends on storage temperature, diversity and numbers of initial microbes, the composition and volume of gaseous atmosphere packaging and the permeability of the pack (Farber, 1991). Under vacuum packaged (VP) or MAP conditions, the goal is to create conditions whereby a subsection of the indigenous microflora out-compete other native micro-organisms on food surfaces and thereby become predominant. Extended shelf life occurs when the predominant bacteria are those with metabolic processes which result in low concentrations of off-odour compounds (Farber, 1991). However, it is frequently difficult to determine which micro-organisms are responsible for producing a specific chemical, especially when end-stage metabolites are generated as the result of metabolic interactions between organisms (Corry, 2007). Russell *et al*. (1995) reported *Shewanella putrefaciens*, *Pseudomonas fluorescens*, and *Pseudomonas fragi* as the predominant organisms recovered from spoiled chicken carcasses stored at 3°C for 15 d.

3.5.1 Poultry meat spoilage by *Pseudomonas* and considerations when using a MAP

The Pseudomonads are motile, psychrotrophic, Gram-negative rods which are mostly aerobic (Liao, 2006). Fluorescent *Pseudomonas* are frequently found in terrestrial and water environments (Arnaut-Rollier *et al*., 1999). Fluorescent *Pseudomonas* dominate the spoilage flora which develops on poultry during low-temperature air storage (Blankenship and Craven, 1982). Spoilage *Pseudomonas* can be isolated on the surface of chicken meat and not greater than 3-4mm into the underlying tissue (Forsyth, 2010). In aerobic conditions, *Pseudomonas* export exopolysaccharides and siderophores which manifests as a fluorescent yellowish-green film on meat in the latter stages of spoilage (Corry, 2007). Arnaut-Rollier *et al.* (1999) reported the predominance of four major *Pseudomonas* groups: *P. fragi*, *P. lundensis,* strains belonging to *P. fluorescens* biovars and an unidentified group of strains that displayed a high similarity to *P. fluorescens* biovars from chicken carcasses stored under chilled aerobic conditions.

High concentrations of CO_2 can inhibit the growth of Gram-negative bacteria such as fluorescent *Pseudomonas* (Sawaya et al., 1995). A reported linear relationship between inhibition of fluorescent *Pseudomonas* and CO_2 exists over a narrow range of gas concentrations. During the refrigerated storage of poultry meat, CO_2 concentrations which exceeded 30% (v/v) were reported as having little additional inhibitory effect on *Pseudomonas* numbers compared with CO_2 concentrations approaching 30% (v/v) (Gill, 1988).

3.5.2 Spoilage by the *Enterobacteriaceae*, their use for process hygiene indication and considerations when using a MAP

The family *Enterobacteriaceae* are a large group of more than twenty species of Gram-negative bacillus some of which are motile (Cowan and Steel, 1993). Various members of the *Enterobacteriaceae* can be found in environments such as water, soil and decomposing plants whereas others inhabit and can cause disease in warm and cold-blooded animals and humans (Forsyth, 2010). The diverse range of natural habitats colonised by the *Enterobacteriaceae* group make them ideal indicators of the hygienic conditions experienced by processed foods. In contrast to psychrotrophic *Pseudomonas*, the *Enterobacteriaceae* predominate during warm storage of chicken meat between 10-20°C (Corry, 2007). It is the *Enterobacteriaceae* that are largely responsible for the sensory odour of packaged chicken (Corry, 2007). Zhang *et al.* (2012) investigated growth of the *Enterobacteriaceae* on air packaged chicken at 4°C, fluctuating between 0-4°C and between 4-10°C. No reduction of numbers was reported under the fluctuating temperatures and the bacteria grew best at the higher 4-10°C range in air. Members of the *Enterobacteriaceae* belonging to several genera have been recovered from meat or meat products after chilled storage including *Enterobacter, Eschericia, Salmonella* and *Yersinia* (Mead, 2007). The growth of some members of the *Enterobacteriaceae* is inhibited by the presence of elevated concentrations of CO_2 when compared with the growth reported in air (Farber, 1991). The most common *Enterobacteriaceae* that causes food spoilage are the lactic acid bacteria (LAB) which are facultative anaerobes. LAB produce lactic acid as a product of carbohydrate fermentation which produces a sour or cheesy odour in contrast to the putrid odour produced by *Pseudomonas* (Corry, 2007).

3.5.3 Alternative methods for the assessment of spoilage

In addition to measuring a number of bacterial indicators, spoilage can also be measured by the colour and odour of meat and its acceptability to consumers. Thiobarbituric acid-reactive substances (TBARS) are routinely analysed as an index of lipid peroxidation and oxidative stress i.e. as degradation products of lipids (Shadbakhsh, 2005). Drip is also a measure of spoilage and is the reason that many packs have a small swab under the meat to absorb excess liquid. Drip formation is a complex phenomenon that is not yet fully understood. Fresh muscle is approximately 75% water (w/w), 80% of which is held in the muscle myofibrillar structure, between the myofibrils, and between the myofibrils and the sarcolemma. Changes that occur in the muscle structure and pH during the transformation of muscle to meat allow water to escape from the muscle as drip (Bowker and Zhuang, 2013).

3.6 MAP and VP as methods for the preservation of poultry meat

Packaging of whatever type is to physically and microbiologically protect meat, prolong shelf-life and maintain organoleptic acceptability. Microbes will multiply on raw meat unless stored frozen (Sawaya *et al*., 1995). During storage, the populations and proportions of the microbes will change with substrate availability and atmospheric conditions including movement to more favourable conditions by motile bacteria as previously discussed. VP does not extend the shelf-life of chicken as effectively as for red meat, and is used less widely for poultry (Corry, 2007). VP allows the growth of the common chicken meat contaminants *Listeria*, lactobacilli and the *Enterobacteriaceae* (Byrd *et al*., 2011) which are less likely to be present on red meat compared with poultry meat (Dainty and Mackey, 1992).

The majority of the chicken fillets for retail sale produced in Denmark are packed in a modified atmosphere containing 70% O_2 and 30% CO_2 (Boysen *et al*., 2007). The approximately 30% upper limit for CO_2 concentration within a MAP is widely used globally by the poultry processing industry because CO_2 is highly soluble in water. If too much CO_2 is absorbed into meat, there is a risk of pack collapse. In addition, there is a chance that the dissolved gas will increase the volume of any free water in the meat, leading to excessive drip (Gill, 1988). These mechanisms are key considerations for packs which are likely to be stored at lower temperatures.

3.6.1 Effect of different MAPs in reducing populations of *Pseudomonas*, the *Enterobacteriaceae* and *Campylobacter* on chicken broiler meat

Table 6 compares data from MAP studies that have used different atmospheres in an attempt to reduce the numbers of *Pseudomonas*, *Enterobacteriaceae* and/or *Campylobacter* during storage. Seventeen studies have been compared, two of which used beef rather than chicken (Dykes and Moorhead, 2001; Hänninen *et al*., 1984). These studies were still included in the review as they provided useful data where few studies are available.

3.6.2 *Pseudomonas* and the *Enterobacteriaceae*

The majority of research undertaken before the year 2000 focused on mixes of CO_2 and N_2 to reduce spoilage bacteria. In the 1990s, Baker *et al*. (1985) reported that naturally contaminated ground chicken meat stored for 7 d in 20% CO_2 and 80% air was as effective at reducing *Pseudomonas* and LAB numbers on chicken meat as higher levels of CO_2. The inhibition was maintained until the 28th day of storage. During the storage, a population shift from the initially predominating *Pseudomonas* to LAB occurred. Broadly in keeping with previous studies, Koulianos (2004) and Brody(1996) reported no significant differences in the inhibition of

Pseudomonas irrespective of whether 20 or 30% CO_2 was included. Woods and Church (1999) reported 25% CO_2 to be most cost-effective at increasing the shelf-life of poultry. This mix of around 25% CO_2 with the remainder being air is used for portions by one of the UK processors.

Sawaya *et al*. (1995) reported that lower temperatures were more effective when using MAP containing CO_2. CO_2 compared with N_2 caused a greater reduction in the numbers of *Pseudomonas* when chicken was stored at 2-4°C compared with 7-9°C. Low temperatures allow more CO_2 to become dissolved into water. The mechanism underlying the low temperature enhancement of otherwise identical MAPs containing 20-30% CO_2 is increased quantities of carbon dioxide being dissolved into water, which increases the concentration of the inhibitor in proximity to the bacteria and additionally lowers the pH of the extracellular liquid (Gill, 1988).

While determining if there was any effect of ozone as part of a MAP, Al-Haddad *et al*. (2005) reported a dramatic fall in numbers of *P. aeruginosa* on chicken stored in a MAP containing 70% CO_2. The chicken was exposed to >2000 ppm ozone for 15 min which reduced *P. aeruginosa* from 94 cfu cm^{-2} (inoculum level) to 1.3 cfu cm^{-2} of chicken skin after 7 d of storage at 7°C. However, a potential issue with the study was that laboratory cultured strains were used to inoculate broiler skin rather than natural contamination. Acknowledging the potential criticism, the authors suggest that a lesser effect of the ozone treatment would be seen were the work to be repeated using naturally contaminated skin. Under those conditions, the contaminating bacteria would better adapt to their environment.

Byrd *et al*. (2011) reported that 100% CO_2 had a significant effect on the multiplication of psychrophiles, slowing an increase in their numbers on chicken meat from 2.58 to 4.21 $\log_{10}$ CFU/ml compared with >6.35 under other treatments during 14 d storage at 2°C (Table 6). Mixes with CO_2 concentrations close to 100% are widely used by the UK poultry industry in mother bags used for bulk storage/transport. One retail pack was also found to contain 85% CO_2. Gas mixes with lower CO_2 concentrations were assessed at 3°C by Byrd *et al*. (2011) (Table 6). As might be expected, the increased temperature and lower CO_2 concentrations tended to support increases to the numbers of psychrophiles. Sawaya *et al*. (1995) reported that high CO_2 (70%) had an inhibitory effect on *Pseudomonas* because the gas mix increases both the lag phase prior to growth and the mean generation time during growth. Similar findings were reported by (Fraqueza and Barreto, 2011) who tested the effect of 50% and 80% CO_2 on raw turkey meat slices (with the balance of the mix volume being filled with N_2). The study observed that CO_2 concentrations over 50% had no additional significant benefit in terms of reduced numbers of psychrophiles and *Enterobacteriaceae* compared with 50% CO_2.

The Fraqueza and Barreto (2011) trial also assessed the impact of adding a small volume of carbon monoxide (CO) at a concentration of 0.5% (v/v). The CO addition did not significantly alter CO_2-mediated inhibition of any of the spoilage bacteria (Fraqueza and Barreto, 2011).

Table 6. Comparison of data from MAP studies using different atmospheres published after 1984

Product	Temp °C	Atmosphere (gas:product)	Storage time (d)	*Campylobacter* log_{10} CFU/mL		*Enterobacteriaceae* log_{10} CFU/mL		*Pseudomonas* log_{10} CFU/mL		Organoleptic	References
				Start	Finish	Start	Finish	Start	Finish		
Skin on breast [J]	7	70% CO_2:30% N_2 unchanged during storage	9	n/a		n/a		[a] 950 cfu/10cm2	400 cfu/10cm2	No smell, dry skin, good colour	(Al-Haddad et al., 2005)
Ground breast and leg, skin on		100% CO_2, balance air 80% 60% 40% 20% 0%	(7) 14			0 (% of total populatio n (APC))	(1) 45 % (1) 50 % (0) 48 % (0) 47 % (0) 40 % (0) 0 %	91 %	(81) 55 %) (80) 50 % (81) 46 % (87) 50 % (81) 55 % (98) 98 %		(Baker et al., 1985)
Bolton broth Fillets	5	70%O_2:30%CO_2 100N_2 70%$N_2$30%CO_2 70%O_2:30%CO_2 70%N_2:30%CO_2 (7)	14 11	log reduction *[r] 1.5-6 [d] 0.3 [d] 0.3 2.2–3.1 no reduction		[b]4.3 [b] 4.1	8.6 8.3			Fillets stored in O_2 retained better pink colour	(Boysen et al., 2007)
Beef steaks	1 5	Vacuum CO_2	14 14	log_{10}CFU/cm^2 * ~5.5-6.5 ~5.5-6.5	No significant reduction No significant reduction						(Dykes and Moorhead, 2001)

Whole chicken car-cass	2 3	100%CO_2 5%O_2:20%CO_2:75%N_2 100%O_2 5%O_2:20%CO_2:75%N_2 100%O_2	14 (2) 14	1.36 1.36 1.36 1.50 1.50	1.00 0.55 0.15 (1.99) 1.83 (1.63) 0.86	[c] 2.36 [c] 2.36 [c] 2.36	[c] 2.03 [c] 2.71 [c] 2.47				(Byrd et al., 2011)
Turkey breast slices	0	50% CO_2:50%N_2 0.5%CO:50%CO_2:49.5%N_2 0.5%CO:80%CO_2:19.5%N_2 100%N_2	14			log10 CFU/g [d] 3	[d] 3.6 [d] 3.2 [d] 2.6 [d] 5.2	[d] 4.5	[d] 5 [d] 4.8 [d] 4 [d] 6	Inclusion of CO improved the retention of the pink colour	(Fraqueza and Barreto, 2011)
Beef 10g pieces	4	80%N_2:20%CO_2 5%O_2:10%CO_2:85%N_2	11	log10 CFU/g *[g] 6	[d] 4.5 4.95						(Hänninen et al., 1984)
Chicken quarters	2	80%CO_2 , balance air 70%CO_2 60% CO_2 Air	(7) 14			[f] 0% total aerobic population	(10) 40 % (0) 0	[d] 3.5	[d] (3.5) 4.1 [d] (3.9) 4.4 [d] (3.6) 4.4	Higher CO_2 produced yellowing skin. No other organolep-tic reduction	(Hotchkiss et al., 1985)
Chicken breast skin off	6	20%CO_2:80%O_2 20%CO_2:60%O_2:20%N_2 20%CO_2:40%O_2:40%N_2 30%CO_2:40%O_2:30%N_2 20%CO_2:10%O_2:70%N_2 20%CO_2:80%N_2	(7) 12	log_{10} CFU/cm^2 *[d] 7.1	[d] (1.2) 0 (2) 0 (4.5) 0.5 (4.3) 0.5 (6.4) 5.5 (7.4) 7.4	[df] 2.8	[df] (4) 6 (4) 6.2 (4.5) 6 (4) 5.8 (4.5) 6 (4.5) 7	[d] 2	[d] (3) 5.8 (3) 7.2 (4.5) 7 (3.1) 5.8 (4.5) 7 (4.5) 7.2	Low O_2 packs had higher off odours than high O_2 packs	(Koulianos, 2004)
Chicken breasts	4	99.5% CO_2:0.5% CO (4:1)	(14) 21	* (6) 6	(6.78) 5.26						(Kudra et al., 2012)
Chicken skin	4	100% CO_2 Microaerophili c N_2	7	log_{10}CFU/ml/cm^2 *7.1 5.6 5.6	No reduction No reduction Increase						(Lee et al., 1998)

Turkey roll	4	100% CO_2 80% CO_2:20% N_2 60% CO_2:40% N_2 40% CO_2:60% N_2 100% N_2 5% O_2:10% CO_2:85% N_2 Air	12	log10 CFU/g *6	[d] 1.9 [d] 1.2 [d] 1.2 [d] <10 [d] <10 [d] <10 [d] 0.7	[f]0.8 [f]0.2 [f]2.2 [f]0.5 [f]1.7 [f]2.8 [f]2.1		[e] <1	2.8 2.9 4.5 3.4 7.9 7.4 8.6		(Phebus et al., 1991)
Chicken legs	4	80% O_2:20% N_2 80% CO_2:20% N_2 (1:1)	5	log_{10} CFU/g *[dh] 2.98 [dh] 2.4	[dh] <1 [dh] 2.2						(Rajkovic et al., 2010)
Chicken carcass	2 4 7	70% CO_2:30% N_2 30% CO_2:70% N_2 70% CO_2:30% N_2 30% CO_2:70% N_2 70% CO_2:30% N_2 30% CO_2:70% N_2	14 7 7			[d] 3.5	[d]4.9 [f]6.6 [d]6.1 [f]7d 6.2 [d]6.2 [f]6.4 [d]7.4 [f]6.4 [d]7 d 6.1 [f]6.9 [d]7 d 7.3 [f]6.2	[d]4.5	[d]5.4 [d]6.5 [d]6.1 [d]6.0 [d]14 d 6.6 [d]14 d 6.1		(Sawaya et al., 1995)
Chicken breasts skin off	4	80% O_2:20% CO_2 70% O_2:30% CO_2 60% O_2:30% CO_2:10% N_2	(6) 13	log_{10} CFU/cm^2 *7	(1.5) 2.1 (4) 1 (3.1) 1.5			3.5	(2.2) 3.9 (2.2) 4.1 (2.2) 4	0.55 TBAR 0.41 TBAR 0.4 TBAR	(Shadbakhsh, 2005)
Campylo-bacter cells	4	80% CO_2:N_2 80% O_2:20% N_2 5% O_2:10% CO_2:85% N_2	7	5	[d] 4.8 [d] 3 d >10 [d] 1						(Smigica et al., 2010)
Chicken carcass, half Ground beef	4 2 . 5	Vacuum O_2 permeable PVC-overlay 100% N_2 Vacuum 80% CO_2:20% N_2 5% O_2:10% CO_2:85% N_2	(2h) 4 (7) 14	*5 Log reduction only reported	[d] (4.9) 4.6 [d] (4.9) 4.5 [d] (0.44) 0.70 [d] (0.7) 1.11 [d] (0.75) 0.95 [d] (0.7) 0.95						(Stern et al., 1986)

* inoculated rather than naturally contaminated
[a] *P. aeruginosa*
[b] *Mesophilic* bacteria
[c] *Escherichia coli* only
[d] data estimated from Figure
[e] psychrotrophic bacteria
[f] LAB
[g] data for strain N 104 isolated from human, not bovine origin or NTCT strains
[h] data for naturally contaminated samples not treated with acid only
[j] also treated with ozone

Air Products (2013) states that ‘levels of CO_2 in excess of 20% are required to significantly extend the shelf-life of raw poultry and game birds. For retail MA packs of raw poultry and game, the proportion of CO_2 in the gas mixture should not be higher than 35% since pack collapse and excessive drip may be induced”. CO_2 levels greater than 30% have been shown to have no further benefit for reducing the rates of *Pseudomonas* multiplication.

3.6.3 Campylobacter

Although it is generally regarded that a natural indigenous microbiota are a better model for MAP studies compared with inoculated strains (Arvanitoyannis and Stratakos, 2012), many studies have used inoculated meat. One of the main drivers for the inoculation studies is that potential human pathogens such as *Salmonella* may have a low prevalence or be present at numbers which are too low to show meaningful reductions. Model systems that have been artificially inoculated may not have representative microbial diversity compared with a natural microflora, bringing into question the relevance of any results (Arvanitoyannis and Stratakos, 2012). *Campylobacter* prevalence in the UK was estimated by EFSA (EFSA, 2010) to be 75% in broiler batches (caecal contents) and on broiler carcasses (skin samples) 86%. Therefore, there is an easily available abundance of naturally contaminated chicken in the EU that could be used for these studies which would provide representative microbial diversity.

Whilst investigating the role of CO_2 in MAP, Phebus *et al*. (1991) inoculated sliced turkey meat with two strains of laboratory-cultured *C. jejuni*. The study assessed the fate of these campylobacters and naturally present spoilage bacteria during storage of the turkey at either 4°C for 18 d or 18°C for 2 d. Seven different gas mixes were assessed by the study (Table 7).

Table 7. Gas mixes used by the Phebus *et al*. (1991) study

Gas mix identifier	Gas mix composition
A	100% CO_2
B	80% CO_2 20% N_2
C	60% CO_2 40% N_2
D	40% CO_2 60% N_2
E	100% N_2
F	100% Air (control mix)
G	5% O_2 10% CO_2 85% N_2

C. jejuni was inactivated when stored under any of the MAPs used in the study at both storage temperatures. Increasing the CO_2 concentration reduced the rates of decline of the campylobacters also at both storage temperatures. With exception of air (mix F), there were significantly greater rates of decline for the microaerophilic MA (mix G) at 4°C when compared to the other gas mixes (Phebus *et al.* 1991). Although a microaerophilic atmosphere is optimum for the survival and multiplication of thermophilic campylobacters at 42°C, the authors discuss the likelihood that at 4°C, the optimum atmospheric composition for survival of campylobacters may be different. Furthermore, as a potential explanation of their unexpected observation, the authors speculate that increased solubility of O_2 at chill temperatures increases dissolved O_2 concentrations which is toxic to the campylobacters. Stern *et al*. (1986) also reported that a microaerophilic atmosphere tended to reduce numbers of campylobacters when compared with a high concentration CO_2 atmosphere at low storage temperatures. For the aerobic, LAB and psychrotrophic spoilage populations on the turkey meat, Phebus *et al*. (1991) summarise their findings as higher CO_2 concentrations provided better inhibition compared to lower CO_2 concentrations.

Over the last 10-15 years, the addition of oxygen into MAPs has become more widespread for commercial food processors. A barrier to the use of O_2 is that it causes oxidative senescence of foods (Air Products, 2013) and requires more complex packaging equipment due to the explosion risk associated with O_2. In laboratory studies, the growth of *Campylobacter* was found to be stopped in mixes using O_2 at concentrations exceeding 10% (Bolton and Coates, 1983).

When the fate of populations of LAB and *Campylobacter* on chicken breast skin were investigated using various O_2 concentrations, populations of both genera were found to reduce at ≥40% O_2 and storage at 6°C (Koulianos, 2004). The latter reported that initial numbers of *Campylobacter* spp. in a 0% O_2 atmosphere stored at 6°C (to replicate imperfectly refrigerated storage) remained almost unchanged. In contrast, when stored in 10% O_2, numbers declined from an initial value of 7.1 to 5.5 log_{10} CFU/cm^2 over an 8 d storage. There were no further declines between 8 d and the end of the experiment at 12 d. Also, *Campylobacter* numbers declined in high oxygen (80%), and the rate of decline was significantly correlated with the concentration of O_2 present. There was no correlation with CO_2 concentration. When chicken breast skin-on and skin-off fillets were stored in ranges of O_2 and CO_2 mixes with 0 to 80% O_2 at 6°C it was concluded that a 70% O_2 and 30 % CO_2 mix provided the best reduction to the numbers of campylobacters across both poultry meat types (Koulianos 2004). The same gas mix was also reported as effective for reducing the multiplication of *Pseudomonas* (Koulianos 2004). A later study by Shadbakhsh (2005) tested skin-off breasts only stored in mixes with 60 to 80% O_2 and concluded that an 80% O_2 and 20% CO_2 mix was optimal, a conclusion broadly in keeping with the Koulianos study. This 80% O_2 and 20% CO_2 is the most widely used mix in the UK. In a broad study which considered more than just MAP, Smigica *et al*. (2010) reported that an O_2 rich MAP was best for reducing *Campylobacter* populations on chicken meat stored at 4°C.

Related work by Melero *et al*. (2012) formed burgers from ground chicken meat inoculated with laboratory-cultured *C. jejuni*. The emphasis of the work was the effect of freezing the inoculated burgers with regard to *L. monocytogenes* and *Campylobacter*. For the unfrozen controls, when the burgers were stored at 4°C in a MAP composed of 50% CO_2 and 50% O_2, the numbers of *Campylobacter* declined below the limit of detection of the quantitative testing method by 2 days. After 2 days, testing switched to enrichment and then sporadic detections for campylobacters were made until day nine. Overall, for inoculated chicken burgers, a MAP composed of 50% CO_2 and 50% O_2 offered no significant advantage over standard air packaging.

3.6.3.1 Motility and adhesion

Boysen *et al*. (2007) stated that *Campylobacter* exposed to O_2 became elongated, less coiled and appeared to lose motility. Motility is potentially an important factor for *Campylobacter* survival because it allows movement to more favourable environments (Blankenship and Craven, 1982). Potential movement of *Campylobacter* to crevices in the packaged chicken to a more favourable redox environment or into the follicles in skin-on products might be important. *Campylobacter* cells were reported by Jang *et al*. (2007) to remain on and in chicken skin in both the spiral and coccoid morphologies. When investigated using confocal laser scanning microscopy, campylobacters were observed to migrate 20- 30 mm inside crevices and feather follicles of the skin. Furthermore, *C. jejuni* in the feather follicles was reported as floating freely in the surrounding liquid, even after the skin had been extensively washed. Incubation at 25°C and 37°C rapidly transformed the *Campylobacter* into the coccoid form. The coccoid form has been associated with a VBNC state (Oliver, 2005). The adhesive ability of the coccoid cells was no different to that of the spiral cells at 25°C and 37°C. Generally, both forms of

Campylobacter had poorer adherence when incubated at 4°C compared with 25 and 37°C. This observation might be important for chilled chicken meat because *Campylobacter* on chickens stored at typical refrigerator temperatures (4°C) might adhere less to the surface of the skin. It is unlikely that campylobacters are motile at 4°C.

Shadbakhsh (2005) investigated the effect of storing chicken breasts skin side up and skin side down in a MAP composed of 80% O_2 and 20% CO_2. There was up to a 6 log_{10} cfu/cm^2 reduction between the numbers of campylobacters reported on the skin side up breasts compared with the skin side down ones. Furthermore, for the skin up meat, no campylobacters were detected after 12 d storage. Shadbakhsh (2005) discusses the possibility that the observed differences may be a consequence of natural short wavelengths of visible and ultraviolet electromagnetic radiation on the *Campylobacter* on the right side up breasts. A role for the motility of *Campylobacter* towards the more favourable conditions between the breast meat and the packaging where lower O_2 levels would occur was also discussed. However, no evidence-based conclusion for the differences between the skin up and down sides of the meat was provided.

3.6.3.2 *Campylobacter* and competition with other indigenous populations

Dykes and Moorhead (2001) discuss whether *Pseudomonas* and other spoilage organisms occupy the same niche as *Campylobacter* on chicken meat and, if that is the case, whether there is competition which will reduce the populations of both spoilage bacteria and pathogens. Whilst it has been reported that *Campylobacter* is a poor competitor in many environments (Farber, 1991), there are also reports that *C. jejuni* was able to effectively compete against populations of spoilage flora that developed during both air and CO_2 atmosphere storage of chicken meat (Blankenship and Craven, 1982). Furthermore there is evidence of cooperative symbiotic relationships on chicken meat surfaces rather than competition. Hilbert *et al*. (2010) studied *Campylobacter* in co-culture with common spoilage organisms in an aerobic atmosphere at 35°C. The conditions were designed to put the campylobacters under oxidative stress. Co-culture of *C. jejuni* with *Proteus mirabilis, Citrobacter freundii, Micrococcus luteus,* and *Enterococcus faecalis* did not result in prolonged survival and multiplication of *C. jejuni* cells under aerobic conditions. However, several *Pseudomonas* species regularly isolated from chicken meat including *P. putida, P. fragi, P. fluorescens*, and *P. chlororaphis* supported the survival and multiplication of *C. jejuni in vitro* under oxidative stress conditions. *P. putida* was particularly adept at supporting campylobacters in air. Electron microscopic investigation of the support mechanism revealed that *C. jejuni* and *P. putida* had interacted by moving into close proximity and generating fibre-like structures which connected both cell types. The exact mechanism by which the fibres supported *Campylobacter* growth was not fully explained by the study. *Campylobacter* isolated from both human clinical samples and chicken meat isolates showed extended survival in the presence of *P. putida*. Campylobacters isolated from broiler faecal samples were much less likely to generate fibre linkages with *P. putida* and did not survive for extended periods in aerobic conditions.

Jang *et al*. (2007) reported that *C. jejuni* cells undergo a morphological change from spiral to coccoid when under oxidative stress. The change in morphology did not occur when *C. jejuni* was co-incubated with a *Pseudomonas* (Hilbert *et al*., 2010). The latter study is interesting because it described a previously unknown mechanism for the survival of campylobacters in aerobic conditions. However, currently there is no information which allows an assessment of the importance of the interactions between *Pseudomonas* and *Campylobacter* to be made in terms of foodborne illness.

Whilst not discussed in any length in this short review, the formation of biofilms might also be important for the survival of *Campylobacter*. Reuter *et al*. (2010) stated that *C. jejuni* biofilms developed more rapidly under oxygen stress (20% O_2) compared with microaerobic conditions (5% O_2 and 10% CO_2). The ability to form a biofilm would increase the likelihood that *Campylobacter* on the lower biofilm layers would survive when exposed to unfavourable redox conditions (Reuter *et al* 2010).

3.7 Non microbiological considerations when using a MAP

Various factors other than microbes affect the shelf-life of chicken meat as described below.

3.7.1 Carbon dioxide and meat pH

Fraqueza and Barreto (2011) noted that the pH of meat decreases as CO_2 concentration increases and speculate that lowered pH contributes to the reduction in spoilage bacteria (data was not presented in the paper). The authors also stated that 'independently of the presence of CO_2, microbial inhibition did not increase linearly with an increase in CO_2 concentration to 80%.' When put in a MAP with a high (60, 70 or 80%) concentration of CO_2, the greatest pH reduction observed was 0.9 pH units for 80% CO_2 MAP.

Hotchkiss *et al.* (1985) reported that *Campylobacter* had a minimum requirement of pH 5.5-5.9 in growth media and that chicken meat surface pH values remained fairly stable at pH 6.01-6.21 in air during chilled storage. In combination, the Hotchkiss and Fraqueza studies make it unlikely that any minor reduction in pH plays a meaningful role in reducing the numbers of campylobacters on chicken meat stored refrigerated in a high CO_2 concentration MAP.

3.7.2 Changes to the atmosphere during storage

Few studies reported on changes to the atmosphere in the packages during storage. Change can occur to the gas composition inside the pack as a consequence of a number of factors including latent metabolism within the meat, microbiological metabolism and changes in gas solubility with temperature (Gill, 1988). In addition, the pack itself may not be gas tight and allow the diffusion of different gasses at different rates (Air Products, 2013). Thus the composition of a MAP is likely to change over time.

In the majority of MAP studies relating to chicken meat, the authors have not monitored the gas composition for the duration of the experiment. However, there are some exceptions. Baker et al. (1985) and Sawaya *et al*. (1995) both report an initial decrease in CO_2 in the MA which eventually stabilised due to water present in the pack having a finite capacity for dissolving CO_2. The compositional changes to the MA reported by Baker *et al*. (1985) were quite dramatic. Two CO_2 concentrations were used for the storage of ground meat. An initial CO_2 concentration of 80% (v/v) decreased to 45% (v/v) over 28 days. When a 40% (v/v) CO_2 concentration was used, it reduced to 20% also after 28 d storage. The majority of this decrease occurred during the first 3-5 days (Sawaya *et al*., 1995). Decreased O_2 concentrations can also occur during refrigerated storage due to latent metabolism of the meat and the use of O_2 by bacterial metabolism (Hänninen *et al*., 1984).

3.7.3 Non microbial spoilage indicators

Most MAP-based studies do not report non-microbiological spoilage considerations such as odour, drip and colour. However, these indicators are very important to the customer and affect the likelihood that a product offered at retail will be sold. Extract release volume (ERV) is used as an indicator of spoilage. The basis of an ERV is that an amount of liquid is released from meat that has been homogenised. The ERV is higher in unspoilt meat and lower in spoilt meat. Sawaya *et al*. (1995) reported the decrease in ERV was concomitant with the proliferation of spoilage bacteria (Table 6).

A yellowing of the skin was noted in chicken samples stored under high CO_2 (Hotchkiss *et al*., 1985).

MAP containing high concentrations of oxygen are popular for red meats because the myoglobin in the meat is converted to oxymyoglobin which has a bright red colour (McMillin, 2008). Although a mixture of 80% O_2 and 20% CO_2 is routinely used in the red meat industry, there are some disadvantages associated with such a mixture (McMillin, 2008). The most important consequence is that unsaturated lipids can auto-oxidise causing flavour deterioration and off-odours. Fatty acids in the meat react with the O_2 in the MA which leads to the formation of fatty acyl hydroperoxides or peroxides and rancid odours. There are a number of factors which influence the rates of conversion of lipids to hydroperoxides or peroxides. These include the fatty acid composition of the meat, presence of pro and anti-oxidants enzymes and storage temperature (McMillin, 2008). There is a lack of information in the published literature relating to off odours and rancidity in chicken meat as a consequence of storage in high O_2 concentration MAs.

Although widely measured for analogous red meat studies, drip volume was not mentioned in any of the reviewed chicken-based literature. It is possible the omission is because drip is principally blood plasma leakage from cut muscles. Cutting is not carried out as often for chicken meat compared with the larger bovine muscles.

3.8 Conclusions

The vast majority of whole birds in the UK were found to be packed in a mix of 80% O_2 and 20% CO_2 or in air. Since this review, some processors have been using a 70%, rather than 80%, oxygen mix. Five gas mixes are used in packing portions and one of these is the 80% O_2 and 20% CO_2 mix. In total, six gas mixes are used for whole birds and portions in the UK.

Based on a review of the pertinent literature, MAP gas mixes that were high in O_2 (~80%) prevented the multiplication of *Enterobacteriaceae* and reduced the numbers of *Campylobacter*. Mixes with moderate (~20%) CO_2 controlled numbers of *Pseudomonas.* A MAP with 80% O_2 and 20% CO_2, as widely used by the UK poultry industry, would be expected to reduce the numbers of campylobacters whilst extending shelf life by reducing the multiplication of key spoilage bacteria.

There are other considerations which include storage temperature, permeability of the package, and gas:meat ratios. Raw chicken is generally chilled and held during storage at 0 to 2°C and then at 4°C during retail display. These temperatures could not be increased and reducing them would be difficult and risk freezing. Low permeability films are already in use. Gas:meat ratios were measured in a later stage of this project (Objective 3).

4. TRIALS TO COMPARE THE EFFECT OF STORAGE IN AIR IN A SEALED PACK WITH STORAGE IN OVERWRAP & TO COMPARE THE EFFECT ON APC OF INCUBATING AT 20°C OR 30°C

4.1 Introduction

The main objective of this FSA-funded project was to define the optimum gas mix to reduce the numbers of *Campylobacter* on chicken carcasses during storage. Some control treatment was required as a reference. One possibility was to store control carcasses in air without any packaging, which could lead to excessive drying and possible airborne contamination.
The aim of this part of the project was therefore to compare the reductions in *Campylobacter* counts when carcasses were packed in air in packaging with high or low gas permeability. The decision would then be made on the type of packaging to be used for storing control samples in later trials.

In addition to *Campylobacter*, testing was also be carried out for aerobic plate counts (APC) and *Pseudomonas*. In the present trial, a comparison is made of the APC resulting from incubation at 20 or 30°C and again a decision would be made as to the conditions to use in further trials.

4.2 Methods

Boot swab samples were taken from ten sheds four days before the expected date of the trial and sent to AFBI for *Campylobacter* testing using real time polymerase chain reaction (RT-PCR). The birds in four sheds were found to be colonised by *Campylobacter* and a batch of birds from one of those sheds was to be processed on its own i.e. not a batch from mixed sheds. The kill plan was known at 16:00 hrs prior to the day of kill. It was hoped that the birds would be processed in the morning, allowing transport to Campden BRI where they would be packed on the same day before further transport to Bristol University for microbiological examination four days later. In practice, the birds were processed much later in the day and came out of the chiller around 20:00 hrs. For that reason, the birds were left overnight in the chilled dispatch area at the processing plant and collected at 05:00 hrs the next morning. They were then transported by chilled courier at 4°C to Campden BRI where they were packed between 09:00 hrs and 11:00 hrs.

A total of 72 birds were used in the trial with 36 birds sealed in bags with a high permeability and 36 birds sealed bags with a low permeability. The high permeability bags (Blender Bags Standard 3500, Grade Packaging Ltd, Coalville, LE67 3FW, UK) had an oxygen transmission rate (OTR) of 2952 to 2693cm^3/m^2/24h and the low permeability bags (PA/PE Bags, The Vacuum Pouch Company, Bury, BL8 3AL, UK) had an oxygen transmission rate (OTR) of 64.5 to 70.0 cm^3/m^2/24h. The OTR was measured using an 8001 oxygen permeation analyzer manufactured by Systech Instruments, Thame, OX9 3XA, UK. These values show a 40-fold difference in the OTRs of the bags. The birds sealed in the low permeability bags were gas flushed with a mix of nitrogen and oxygen in the same ratio as air using a Multivac C200 and A300 Pouch Sealer (Multivac, Swindon, SN5 7UY, UK). The birds sealed in the high permeability bags were not gas flushed as this simulated overwrapping where gas flushing would not be used. The bags were sealed with a hand operated bench impulse sealer (Hulme-Martin, Woking, GU24 9LZ, UK). The gas:meat ratio was 0.5 as this had been previously determined to be used widely by the industry for packing of whole birds. The birds were then transported to Bristol University within 2 hours where they were held for 4 days in a chiller at 4°C.

Breast skin samples from the birds were tested to determine numbers of campylobacters, *Pseudomonas*, and total viable counts of aerobic bacteria (APC). The use of breast skins had been agreed with the FSA Monitoring prior to the start of the project. Breast skin was chosen because it is exposed to the MAP headspace in the pack and because there is sufficient breast skin to sample. Neck skins tend to be wrapped under the carcass and perhaps not exposed to the gas. Also, neck skins tend to be cut very short requiring skin from other parts to be included in the sample in order to obtain sufficient material for testing. Thirty-six skin sample replicates were used for each packaging method.

For campylobacters, four volumes of maximum recovery diluent (MRD) were added to a stomacher bag containing 20g of excised breast skin. The bag contents were homogenised for 1 minute using a stomacher (model BA 6021, Seward, Worthing, UK). The resultant diluent was used for the enumeration of all of the bacteria as follows. For campylobacters, 200 μl of the 1:5 initial dilution was plated onto modified charcoal cefoperazone deoxycholate agar plates (CCDA, Oxoid) in duplicate. All subsequent dilutions were decimal and used 100 μl volumes. Incubation was under microaerobic conditions (CampyGen, Oxoid) at 41.5^{o}C for 48 h. Confirmation of *Campylobacter* spp. was by microscopic examination to confirm corkscrew motility, oxidase activity and serology (DrySpot *Campylobacter*, Oxoid).

The initial homogenate was diluted further to 1:10 and used for the determination of numbers of total viable counts of aerobic bacteria (APC) and *Pseudomonas*. Further dilutions were decimal. APC were determined by standard plate count methods according to the criteria specified by ISO 4833 (2003). In brief, sample homogenates from 1:10 were diluted decimally in MRD, and 1-ml aliquots were added to appropriately labelled petri dishes. Tempered (46^{o}C) plate count agar (15ml; Oxoid, Basingstoke, UK) was added to each petri dish, mixed, and allowed to harden. To determine the most appropriate incubation temperature, duplicate sets of APC plates were prepared for incubation at either 30^{o}C or 20^{o}C for 72 h before the colonies were counted.

Pseudomonas numbers present in the stomached diluent were determined by direct plating onto *Pseudomonas* agar base (Oxoid) supplemented with CFC selective agar supplement (10 mg/liter cetrimide, 10 mg/liter fucidin, and 50 mg/liter cephaloridine; Oxoid) at 25^{o}C for 48 h. Bacterial numbers on all decimally-diluted plates were converted into colony forming units (cfu) per gram according to the criteria described in ISO 6887-1 (1999).

4.3 Results

Tables 8a and 8b, and Figure 1, show the microbial counts on the breast skin samples after storage for 4 days. There was significant evidence of a small effect of differences in the packaging on APCs on samples incubated at 20°C (difference = 0.2 log cfu per g; p=0.042) and no evidence of an effect of the packaging when samples were incubated at 30°C (p=0.241). Similarly, there was no evidence of an effect of differences in the packaging on the numbers of *Pseudomonas* (p=0.841). However, there was strong evidence that the packaging did affect the numbers of campylobacters (difference = 0.36 log cfu per g; p=0.001). Although some of the counts of *Campylobacter* were below the limit of detection of 12.5 cfu per g (replaced by $12.5/\sqrt{2}$ in Tables 8a and 8b), the results show that birds in air-flushed packs with low permeability were greater than the counts on packs with higher permeability. Higher film permeability, when using air, resulted in lower *Campylobacter* counts.

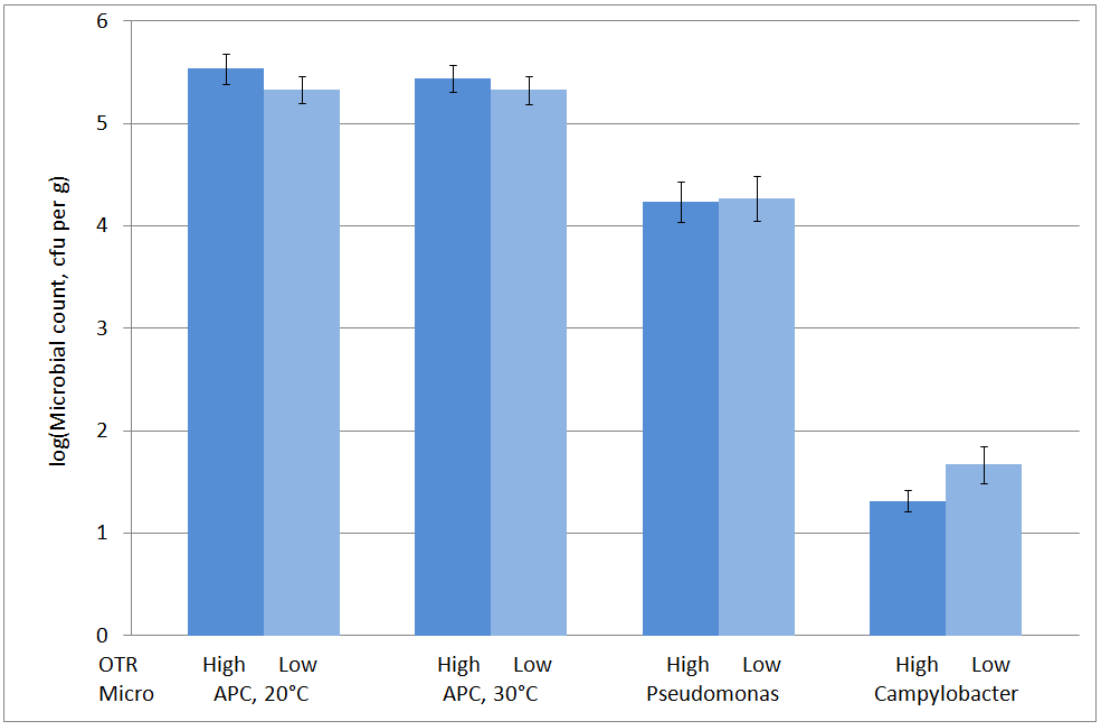
Table 8a. Microbial counts (APC, *Pseudomonas* and *Campylobacter*) on breast skin samples from birds stored for 4 days in high permeability (2952 to 2693 $cm^3/m^2/24h$) bags containing air

Sample no.	Oxygen transmission rate, $cm^3/m^2/24h$	Aerobic Plate Count cfu/g (20°C)	log(APC) (20°C)	Aerobic Plate Count cfu/g (30°C)	log(APC) (30°C)	Pseudomonas cfu/g	log(Pseuds)	Campylobacter cfu/g	log(Campy)
1	High	140000	5.15	260000	5.41	20000	4.30	12.5	1.10
2	High	210000	5.32	100000	5.00	10000	4.00	<12.5	0.95
3	High	70000	4.85	80000	4.90	3960	3.60	12.5	1.10
4	High	120000	5.08	130000	5.11	5545	3.74	62.5	1.80
5	High	850000	5.93	630000	5.80	70000	4.85	<12.5	0.95
6	High	230000	5.36	210000	5.32	30000	4.48	<12.5	0.95
7	High	90000	4.95	240000	5.38	4752	3.68	37.5	1.57
8	High	120000	5.08	140000	5.15	50000	4.70	50	1.70
9	High	540000	5.73	260000	5.41	10000	4.00	<12.5	0.95
10	High	280000	5.45	220000	5.34	30000	4.48	50	1.70
11	High	160000	5.20	110000	5.04	10000	4.00	<12.5	0.95
12	High	360000	5.56	200000	5.30	6100	3.79	<12.5	0.95
13	High	320000	5.51	260000	5.41	10000	4.00	25	1.40
14	High	970000	5.99	370000	5.57	20000	4.30	25	1.40
15	High	710000	5.85	380000	5.58	30000	4.48	12.5	1.10
16	High	620000	5.79	190000	5.28	3600	3.56	12.5	1.10
17	High	320000	5.51	280000	5.45	20000	4.30	62.5	1.80
18	High	480000	5.68	420000	5.62	30000	4.48	37.5	1.57
19	High	280000	5.45	350000	5.54	80000	4.90	12.5	1.10
20	High	480000	5.68	460000	5.66	10000	4.00	25	1.40
21	High	180000	5.26	170000	5.23	3900	3.59	25	1.40
22	High	440000	5.64	330000	5.52	80000	4.90	50	1.70
23	High	410000	5.61	1020000	6.01	20000	4.30	75	1.88
24	High	50000	4.70	40000	4.60	5300	3.72	12.5	1.10
25	High	540000	5.73	470000	5.67	20000	4.30	12.5	1.10
26	High	70000	4.85	90000	4.95	2400	3.38	37.5	1.57
27	High	110000	5.04	30000	4.48	20000	4.30	12.5	1.10
28	High	460000	5.66	360000	5.56	80000	4.90	25	1.40
29	High	3040000	6.48	1840000	6.26	10000	4.00	50	1.70
30	High	220000	5.34	290000	5.46	10000	4.00	37.5	1.57
31	High	740000	5.87	610000	5.79	10000	4.00	37.5	1.57
32	High	360000	5.56	190000	5.28	1200	3.08	37.5	1.57
33	High	3120000	6.49	2160000	6.33	340000	5.53	<12.5	0.95
34	High	1880000	6.27	1060000	6.03	360000	5.56	12.5	1.10
35	High	290000	5.46	270000	5.43	10000	4.00	12.5	1.10
36	High	1230000	6.09	750000	5.88	170000	5.23	<12.5	0.95
Average			5.53		5.44		4.23		1.31
S.D.			0.43		0.40		0.57		0.31
C.I.			0.15		0.14		0.19		0.10
N			36		36		36		36
n<12.5									8

Table 8b. Microbial counts (APC, *Pseudomonas* and *Campylobacter*) on breast skin samples on birds stored for 4 days in low permeability (64.5 to 70.0 $cm^3/m^2/24h$) bags containing air

Sample no.	Oxygen transmission rate, $cm^3/m^2/24h$	Plate Count cfu/g (20°C)	log(APC) (20°C)	Aerobic Plate Count cfu/g (30°C)	log(APC) (30°C)	Pseudomonas cfu/g	log(Pseuds)	Campylobacter cfu/g	log(Campy)
37	Low	170000	5.23	250000	5.40	10000	4.00	12.5	1.10
38	Low	290000	5.46	180000	5.26	10000	4.00	<12.5	0.95
39	Low	190000	5.28	260000	5.41	10000	4.00	12.5	1.10
40	Low	120000	5.08	220000	5.34	20000	4.30	25	1.40
41	Low	190000	5.28	90000	4.95	30000	4.48	137.5	2.14
42	Low	570000	5.76	360000	5.56	50000	4.70	87.5	1.94
43	Low	490000	5.69	820000	5.91	40000	4.60	37.5	1.57
44	Low	1580000	6.20	860000	5.93	110000	5.04	150	2.18
45	Low	460000	5.66	260000	5.41	70000	4.85	150	2.18
46	Low	60000	4.78	1220000	6.09	30000	4.48	<12.5	0.95
47	Low	190000	5.28	200000	5.30	10000	4.00	262.5	2.42
48	Low	450000	5.65	380000	5.58	50000	4.70	37.5	1.57
49	Low	1310000	6.12	880000	5.94	720000	5.86	12.5	1.10
50	Low	240000	5.38	200000	5.30	80000	4.90	237.5	2.38
51	Low	220000	5.34	220000	5.34	10000	4.00	12.5	1.10
52	Low	90000	4.95	120000	5.08	40000	4.60	50	1.70
53	Low	220000	5.34	140000	5.15	60000	4.78	50	1.70
54	Low	340000	5.53	300000	5.48	50000	4.70	237.5	2.38
55	Low	60000	4.78	20000	4.30	1100	3.04	<12.5	0.95
56	Low	100000	5.00	40000	4.60	60000	4.78	25	1.40
57	Low	180000	5.26	120000	5.08	20000	4.30	75	1.88
58	Low	50000	4.70	50000	4.70	10000	4.00	125	2.10
59	Low	250000	5.40	50000	4.70	20000	4.30	100	2.00
60	Low	100000	5.00	210000	5.32	600	2.78	25	1.40
61	Low	280000	5.45	340000	5.53	10000	4.00	175	2.24
62	Low	50000	4.70	80000	4.90	10000	4.00	712.5	2.85
63	Low	450000	5.65	300000	5.48	30000	4.48	25	1.40
64	Low	30000	4.48	120000	5.08	20000	4.30	112.5	2.05
65	Low	1000000	6.00	870000	5.94	400	2.60	350	2.54
66	Low	180000	5.26	170000	5.23	20000	4.30	12.5	1.10
67	Low	200000	5.30	200000	5.30	50000	4.70	62.5	1.80
68	Low	330000	5.52	390000	5.59	20000	4.30	25	1.40
69	Low	330000	5.52	350000	5.54	10000	4.00	50	1.70
70	Low	150000	5.18	170000	5.23	800	2.90	25	1.40
71	Low	480000	5.68	600000	5.78	50000	4.70	<12.5	0.95
72	Low	100000	5.00	100000	5.00	10000	4.00	12.5	1.10
Average			5.33		5.33		4.26		1.67
S.D.			0.39		0.40		0.65		0.53
C.I.			0.13		0.14		0.22		0.18
N			36		36		36		36
n<12.5									4

Figure 1. Microbial counts (APC, *Pseudomonas* and *Campylobacter*) on breast skin samples stored for 4 days in packaging with high oxygen transmission rate (OTR = 2952 to 2693 $cm^3/m^2/24h$) or low OTR bags containing air. Aerobic plate counts were incubated at 20 or 30°C



4.4 Discussion and conclusions

The permeability of the packaging affected the numbers of *Campylobacter* on the samples with higher permeability resulting in lower counts of *Campylobacter*. After the trial, some chickens in sealed non-MAP packs were obtained from a local retailer. Two types of whole bird were obtained (Standard and Saver). The OTR of the packaging films were between 14471 and 15638 $cm^3/m^2/24h$ (Standard) and 8253 to 11550 $cm^3/m^2/24h$ (Saver). The use of similar film with high permeability was proposed for use as the control in further work as the results from this trial showed that high permeability reduced *Campylobacter* numbers.
Samples would be incubated at 30°C for APC testing in further trials.

5. TRIALS TO MEASURE GAS:MEAT RATIOS (OBJECTIVE 3)

5.1 Introduction

The review of the effect of MAP on *Campylobacter* found no peer-reviewed papers that provided data on gas:meat ratios. Information was found in the literature from two of the gas suppliers (Air Products, 2004 and Linde Group, 2012 and 2013) both recommending gas:meat ratios for chicken of 2:1 although one of the Linde Group (2012) pamphlets suggests 0.02 to 0.04 cubic feet of air per lb of meat. Assuming a density of meat of 1000 kg m^{-3} (62.4 lb ft^{-3}) gives estimated gas:meat ratios of 1.2 to 2.5.

This report describes measurements of gas:meat ratio of packs of whole chickens and portions obtained from two processors. Tests were carried out on groups of 5 samples of each product that included 5 whole bird formats and 11 portion formats.

5.2 Methods

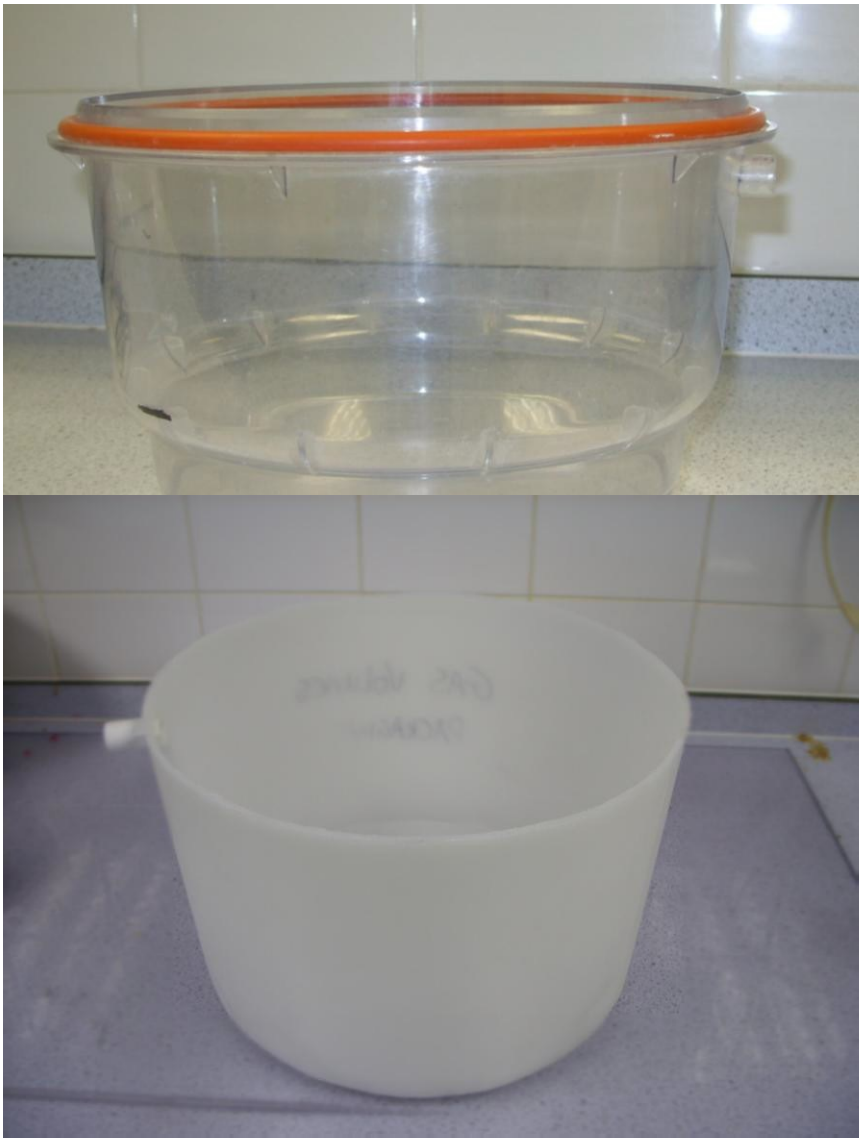
The volume of a pack containing poultry (whole bird or portions) was measured by filling a container of water to the level of the spout (Figure 2) and then placing the packed chicken in to the water. The small container was used with small packs and the larger container with whole birds. The volume of the overflow of water escaping from the spout was measured.

The volume of gas in the pack was then measured by submerging the pack of poultry underwater along with a measuring cylinder. The measuring cylinder was filled with water and then placed (inverted) over the immersed pack of poultry. A hole was made in the packaging of the poultry with the cylinder over the hole to catch any gas that escaped. The pack and poultry were squeezed to aid the flow of gas from the pack into the measuring cylinder. The volume of water displaced from the cylinder (equal to the gas volume) was then measured in the cylinder.

Once the gas volume has been recorded, the poultry was taken out of the packaging to record the weight and volume of the poultry itself. The latter was carried out in the same way as step 1 by filling the container to the spout level, placing the poultry in the water, and recording the volume of water displaced.

Finally, in the last stage, the volume of the packaging was measured by putting 500ml of water into a 1000ml measuring cylinder. The packaging was then cut into pieces and placed into the cylinder and pushed down into the water using a brush. The volume of the packaging was then recorded as the increase in level of water in the measuring cylinder.

Figure 2. Photographs of containers with spouts used to measure the volumes of packs and poultry



Five packs of each format were treated in the way just described. The concentration of gas in one pack of each format was measured by inserting a hypodermic syringe into the pack and the gas analysed using a Systech Gaspace analyser (Systech Instruments, Thame, OX9 3XA, UK). The small hole was covered with an adhesive pad prior to the measurement of pack volume. The volume of gas removed for analysis was insignificant and no significant differences were found in the volumes of packs that had or had not been used for gas analysis.

The following data were recorded: weight of the poultry recorded on the pack (W, kg), details of the product recorded on the pack, volume of whole pack (V_{wp}, ml), volume of the gas in the pack assessed using the measuring cylinder (V_g), weight of the meat (W_m, kg), volume of unpacked poultry alone (V_c, ml), and volume of the packaging alone (V_p, ml).

The gas:meat ratios were calculated in two ways:

$$\text{Gas:meat ratio Estimate 1} = \frac{\text{Volume of gas in the pack } (V_g)}{\text{Volume of chicken alone } (V_c)}$$

$$\text{Gas:meat ratio Estimate 2} = \frac{\text{Volume of unopened pack } (V_{wp}) - \text{Volume of chicken alone } (V_c) - \text{Volume of packaging } (V_p)}{\text{Volume of chicken alone } (V_c)}$$

The two methods measured different factors. Estimate 1, using the gas cylinder, measured the volume of gas at atmospheric pressure. Estimate 2, which included a measurement of the pack volume, measured the volume of gas at the internal pack pressure. Provided that the pack pressure is close to atmospheric pressure, shown by the pack not being domed upwards or downwards, then the two estimates should be similar. However, Estimate 1 might be lower due to the difficulty in removing gas from within the cavity and crevices of the poultry carcasses. There was also the possibility that the poultry would absorb water but this was unlikely in the short period that the poultry was immersed in the water.

5.3 Results

Trials were carried out on three days and on each day packs were obtained from the packing plant and assessments of gas:meat ratios made on the day of packing. However, processors often move poultry from one plant to another for cutting and packing operations so the date of packing relative to kill date was recorded.

Tables 9a to 9f, 10a-10d, and 11a-11f show the individual measurements from each of the three trials. Generally, the two approaches to measuring gas:meat ratios gave close agreement with the maximum difference between the two approaches being 0.35 (based on average of 5 values for a given product/pack format).

Table 9a. Estimates of gas:meat ratios of five packs of skin-on chicken thighs from Processor A

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
A1	Thigh	K+1	1				2040	940	1.033	1065	45	0.88	0.87	0.88	-0.01	
A2	Thigh	K+1	1				2060	945	1.060	1040	35	0.91	0.95	0.93	0.04	
A3	Thigh	K+1	1				2110	960	1.130	1140	40	0.84	0.82	0.83	-0.03	
A4	Thigh	K+1	1				1980	820	1.192	1110	45	0.74	0.74	0.74	0.00	
A5	Thigh	K+1	1	27.2	16.2	56.6	2090	910	1.133	1060	45	0.86	0.93	0.89	0.07	
Average							2056	915	1.110	1083	42	0.85	0.86	0.85	0.02	0.85
S.D.							50	56	0.063	41	4	0.07	0.08			0.07
C.I.							70	78	0.088	57	6	0.09	0.12			0.05
Max							2110	960	1.192	1140	45	0.91	0.95			0.95
Min							1980	820	1.033	1040	35	0.74	0.74			0.74

Table 9b. Estimates of gas:meat ratios of five packs of skin-on chicken drumsticks from Processor A

Pack No.	Format (thigh, leg etc)		Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	V_g/V_c	$(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
B1	Drumstick	K+1	0.5				1490	840	0.571	530	30	1.58	1.75	1.67	0.17	
B2	Drumstick	K+1	0.5				1460	795	0.546	490	30	1.62	1.92	1.77	0.30	
B3	Drumstick	K+1	0.5				1525	775	0.594	530	30	1.46	1.82	1.64	0.36	
B4	Drumstick	K+1	0.5				1505	730	0.570	650	35	1.12	1.26	1.19	0.14	
B5	Drumstick	K+1	0.5	23	24.1	52.9	1450	810	0.585	520	35	1.56	1.72	1.64	0.16	
Average							1486	790	0.573	544	32	1.47	1.70	1.58	0.23	1.58
S.D.							31	41	0.018	61	3	0.20	0.25			0.25
C.I.							43	57	0.025	85	4	0.28	0.35			0.19
Max							1525	840	0.594	650	35	1.62	1.92			1.92
Min							1450	730	0.546	490	30	1.12	1.26			1.12

Table 9c. Estimates of gas:meat ratios of five packs of skin-off chicken mini-fillets from Processor A

Pack No.	Format (thigh, leg etc)		Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	V_g/V_c	$(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
C1	Mini Fillet	K+2	0.25				555	335	0.264	220	15	1.52	1.45	1.49	-0.07	
C2	Mini Fillet	K+2	0.25				600	320	0.267	230	15	1.39	1.54	1.47	0.15	
C3	Mini Fillet	K+2	0.25				570	310	0.269	240	15	1.29	1.31	1.30	0.02	
C4	Mini Fillet	K+2	0.25				525	300	0.261	220	10	1.36	1.34	1.35	-0.02	
C5	Mini Fillet	K+2	0.25	84.5	13	2.5	500	290	0.252	230	10	1.26	1.13	1.20	-0.13	
Average							550	311	0.263	228	13	1.37	1.36	1.36	-0.01	1.36
S.D.							39	17	0.007	8	3	0.10	0.16			0.12
C.I.							54	24	0.009	12	4	0.14	0.22			0.09
Max							600	335	0.269	240	15	1.52	1.54			1.54
Min							500	290	0.252	220	10	1.26	1.13			1.13

Table 9d. Estimates of gas:meat ratios of five packs of skin-on chicken breast fillets from Processor A

Pack No.	Format (thigh, leg etc)		Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	V_g/V_c	$(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
D1	Skin Breast	K+2	0.382				610	305	0.389	380	20	0.80	0.55	0.68	-0.25	
D2	Skin Breast	K+2	0.317				600	400	0.322	300	15	1.33	0.95	1.14	-0.38	
D3	Skin Breast	K+2	0.351				585	350	0.356	325	20	1.08	0.74	0.91	-0.34	
D4	Skin Breast	K+2	0.403				640	290	0.405	360	20	0.81	0.72	0.76	-0.08	
D5	Skin Breast	K+2	0.354	86.9	10.4	2.7	655	310	0.362	350	20	0.89	0.81	0.85	-0.07	
Average							618	331	0.367	343	19	0.98	0.76	0.87	-0.23	0.87
S.D.							29	44	0.032	31	2	0.23	0.14			0.21
C.I.							40	62	0.044	43	3	0.31	0.20			0.16
Max							655	400	0.405	380	20	1.33	0.95			1.33
Min							585	290	0.322	300	15	0.80	0.55			0.55

Table 9e. Estimates of gas:meat ratios of four packs of skin-off chicken breast fillets from Processor A. Pack E3 burst during handling.

Pack No.	Format (thigh, leg etc)		Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	V_g/V_c	$(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 9 values
E1	Breast	K+2	0.65				1240	540	0.648	590	35	0.92	1.04	0.98	0.13	
E2	Breast	K+2	0.65				1255	600	0.655	620	30	0.97	0.98	0.97	0.01	
E3	Breast	K+2	0.65				1180	*Split 350	0.660	610	35		0.88	0.88		
E4	Breast	K+2	0.65				1160	550	0.662	610	40	0.90	0.84	0.87	-0.07	
E5	Breast	K+2	0.65	89.9	10.7	-0.6	1280	510	0.656	600	35	0.85	1.08	0.96	0.23	
Average							1223	550	0.656	606	35	0.91	0.96	0.93	0.07	0.94
S.D.							51	37	0.005	11	4	0.05	0.10			0.08
C.I.							71	52	0.008	16	5	0.09	0.14			0.06
Max							1280	600	0.662	620	40	0.97	1.08			1.08
Min							1160	510	0.648	590	30	0.85	0.84			0.84

Table 9f. Estimates of gas:meat ratios of five packs of chicken whole birds from Processor A

Pack No.	Format (thigh, leg etc)		Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	V_g/V_c	$(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
F1	Whole	K					2120	610	1.447	1350	25	0.45	0.55	0.50	0.10	
F2	Whole	K					2175	620	1.582	1500	25	0.41	0.43	0.42	0.02	
F3	Whole	K					2180	680	1.416	1330	20	0.51	0.62	0.57	0.11	
F4	Whole	K					2290	650	1.579	1470	25	0.44	0.54	0.49	0.10	
F5	Whole	K		80.3	14.3	5.4	2390	580	1.614	1535	25	0.38	0.54	0.46	0.16	
Average							2231	628	1.528	1437	24	0.44	0.54	0.49	0.10	0.49
S.D.							108	38	0.089	92	2	0.05	0.07			0.08
C.I.							150	53	0.124	127	3	0.07	0.09			0.06
Max							2390	680	1.614	1535	25	0.51	0.62			0.62
Min							2120	580	1.416	1330	20	0.38	0.43			0.38

Table 10a. Estimates of gas:meat ratios of five packs of diced breast fillets from Processor B

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
A1	Diced Breast	K+1	0.4	83.6	14.9	1.5	985	490	0.426	375	25	1.31	1.56	1.43	0.25	
A2	Diced Breast	K+1	0.4				975	420	0.420	425	30	0.99	1.22	1.11	0.24	
A3	Diced Breast	K+1	0.4				925	490	0.412	385	30	1.27	1.32	1.30	0.05	
A4	Diced Breast	K+1	0.4				965	420	0.412	460	25	0.91	1.04	0.98	0.13	
A5	Diced Breast	K+1	0.4				860	440	0.433	410	25	1.07	1.04	1.05	-0.04	
Average							942	452	0.421	411	27	1.11	1.24	1.17	0.13	1.17
S.D.							51	36	0.009	34	3	0.17	0.22			0.20
C.I.							71	49	0.013	47	4	0.24	0.30			0.15
Max							985	490	0.433	460	30	1.31	1.56			1.56
Min							860	420	0.412	375	25	0.91	1.04			0.91

Table 10b. Estimates of gas:meat ratios of five packs of skin-on drumsticks from Processor B. NL=Not labelled

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
B1	Drumstick	K+1	NL	82.3	14.6	3.1	2030	665	1.230	1150	35	0.58	0.73	0.66	0.16	
B2	Drumstick	K+1	NL				2070	810	1.071	950	35	0.85	1.14	1.00	0.29	
B3	Drumstick	K+1	NL				2030	730	1.106	1015	35	0.72	0.97	0.84	0.25	
B4	Drumstick	K+1	NL				2170	750	1.160	1070	35	0.70	1.00	0.85	0.29	
B5	Drumstick	K+1	NL				2075	870	1.151	1090	30	0.80	0.88	0.84	0.08	
Average							2075	765	1.144	1055	34	0.73	0.94	0.84	0.21	0.84
S.D.							57	78	0.060	76	2	0.10	0.15			0.17
C.I.							79	109	0.084	105	3	0.15	0.21			0.13
Max							2170	870	1.230	1150	35	0.85	1.14			1.14
Min							2030	665	1.071	950	30	0.58	0.73			0.58

Table 10c. Estimates of gas:meat ratios of five packs of skin-off breasts from Processor B. NL=Not labelled

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
C1	Breast	K+1	NL	81.8	16	2.2	1220	480	0.730	670	30	0.72	0.78	0.75	0.06	
C2	Breast	K+1	NL				1260	490	0.711	660	30	0.74	0.86	0.80	0.12	
C3	Breast	K+1	NL				1260	480	0.669	620	30	0.77	0.98	0.88	0.21	
C4	Breast	K+1	NL				1300	510	0.690	660	30	0.77	0.92	0.85	0.15	
C5	Breast	K+1	NL				1330	470	0.700	650	30	0.72	1.00	0.86	0.28	
Average							1274	486	0.700	652	30	0.75	0.91	0.83	0.16	0.83
S.D.							42	15	0.023	19	0	0.03	0.09			0.11
C.I.							59	21	0.032	27	0	0.04	0.13			0.08
Max							1330	510	0.730	670	30	0.77	1.00			1.00
Min							1220	470	0.669	620	30	0.72	0.78			0.72

Table 10d. Estimates of gas:meat ratios of five packs of skin-on legs from Processor B. NL=Not labelled

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est 2	Difference Est2-Est1	Average based on 10 values
D1	Leg	K+1	NL	81.8	16.4	1.8	2140	850	1.012	965	35	0.88	1.18	1.03	0.30	
D2	Leg	K+1	NL				2110	810	0.970	915	30	0.89	1.27	1.08	0.39	
D3	Leg	K+1	NL				2120	820	0.948	885	35	0.93	1.36	1.14	0.43	
D4	Leg	K+1	NL				2080	830	1.020	950	35	0.87	1.15	1.01	0.28	
D5	Leg	K+1	NL				2115	890	1.006	940	35	0.95	1.21	1.08	0.27	
Average							2113	840	0.991	931	34	0.90	1.24	1.07	0.33	1.07
S.D.							22	32	0.031	32	2	0.03	0.08			0.18
C.I.							30	44	0.043	44	3	0.04	0.11			0.14
Max							2140	890	1.020	965	35	0.95	1.36			1.36
Min							2080	810	0.948	885	30	0.87	1.15			0.87

Table 11a. Estimates of gas:meat ratios of five packs of whole birds (Large A) from Processor B

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est2	Difference Est2-Est1	Average based on 10 values
A1	WB Large A	K+1	NL	15.9	20	64.1	2700	610	1948	1830	25	0.33	0.46	0.40	0.13	
A2	WB Large A	K+1	NL				2920	820	1807	1710	25	0.48	0.69	0.59	0.21	
A3	WB Large A	K+1	NL				2960	800	1872	1740	30	0.46	0.68	0.57	0.22	
A4	WB Large A	K+1	NL				2700	640	1770	1705	25	0.38	0.57	0.47	0.19	
A5	WB Large A	K+1	NL				2560	550	1781	1660	30	0.33	0.52	0.43	0.19	
Average							2768	684	1835.6	1729	27	0.40	0.59	0.49	0.19	0.49
S.D.							168	120	74	63	3	0.07	0.10			0.13
C.I.							208	149	92	79	3	0.09	0.13			0.09
Max							2960	820	1948	1830	30	0.48	0.69			0.69
Min							2560	550	1770	1660	25	0.33	0.46			0.33

Table 11b. Estimates of gas:meat ratios of five packs of whole birds (Small B) from Processor B

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est2	Difference Est2-Est1	Average based on 10 values
B1	WB Small B	K+1	NL	15.5	23.7	60.8	1930	730	990	930	15	0.78	1.06	0.92	0.27	
B2	WB Small B	K+1	NL				2200	850	1149	1080	20	0.79	1.02	0.90	0.23	
B3	WB Small B	K+1	NL				2150	830	1063	1002	20	0.83	1.13	0.98	0.30	
B4	WB Small B	K+1	NL				2150	780	1137	1075	15	0.73	0.99	0.86	0.26	
B5	WB Small B	K+1	NL				2120	635	926	880	15	0.72	1.39	1.06	0.67	
Average							2110	765	1053	993.4	17	0.77	1.12	0.94	0.35	0.94
S.D.							105	86	95	88	3	0.05	0.16			0.21
C.I.							130	107	119	110	3	0.06	0.20			0.15
Max							2200	850	1149	1080	20	0.83	1.39			1.39
Min							1930	635	926	880	15	0.72	0.99			0.72

Table 11c. ***Estimates of gas:meat ratios of five packs of whole birds (Large C) from Processor B.*** **NL – Not labelled; NR – Not recorded; * Package leaking, small volume of gas escaped;** ** Tray broken and film split; *** Missing values due to leak or split

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 (V_{wp}-V_c-V_p)/V_c	Average of Est1 and Est2	Difference Est2-Est1	Average based on 7 values
C1	WB Large C	K+1	NR	82.8	11.6	5.6	2725	500	1981	1885	35	0.27	0.43	0.35	0.16	
C2	WB Large C	K+1	NR				2980	640	2137	2040	35	0.31	0.44	0.38	0.13	
C3	WB Large C	K+1	NR				3000	600	2145	2050	30	0.29	0.45	0.37	0.16	
C4	WB Large C	K+1	NR				2800	465*	2041	1955	35	***	0.41	***	***	
C5	WB Large C	K+1	NR				**	**	**	**	**	**	**	**	**	
Average							2876	580	2076	1983	34	0.29	0.43	0.37	0.14	0.37
S.D.							135	72	79	78	3	0.02	0.02			0.08
C.I.							215	179	126	124	4	0.06	0.03			0.07
Max							3000	640	2145	2050	35	0.31	0.45			0.45
Min							2725	500	1981	1885	30	0.27	0.41			0.27

Table 11d. Estimates of gas:meat ratios of five packs of whole birds (Small D) from Processor B. NL – Not labelled; NR – Not recorded; * Package leaking, small volume of gas escaped; ** Tray broken and film split; *** Missing values due to leak or split

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 (V_{wp}-V_c-V_p)/V_c	Average of Est1 and Est2	Difference Est2-Est1	Average based on 8 values
D1	WB Small D	K+1	NR	79.6	11.5	8.9	1600	400	1160	1115	5	0.36	0.43	0.39	0.07	
D2	WB Small D	K+1	NR				1540	480	1060	1000	5	0.48	0.54	0.51	0.06	
D3	WB Small D	K+1	NR				1550	430*	1052	975	5	*	0.58	*	*	
D4	WB Small D	K+1	NR				1700	455*	1181	1100	5	*	0.54	*	*	
D5	WB Small D	K+1	NR				1550	500	1081	1015	5	0.49	0.52	0.51	0.03	
Average							1588	460	1106.8	1041	5	0.44	0.52	0.47	0.08	0.49
S.D.							67	53	60	63	0	0.07	0.06			0.07
C.I.							83	66	74	78	0	0.18	0.07			0.06
Max							1700	500	1181	1115	5	0.49	0.58			0.58
Min							1540	400	1052	975	5	0.36	0.43			0.36

Table 11e. Estimates of gas:meat ratios of five packs of mini fillets from Processor B

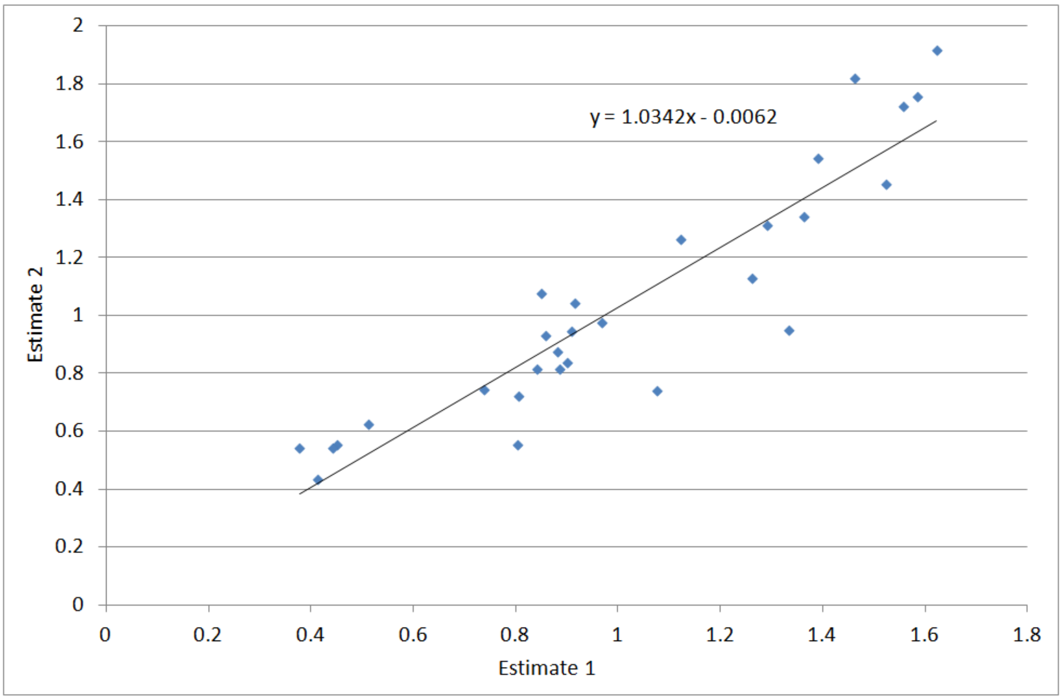
Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est2	Difference Est2-Est1	Average based on 10 values
E1	Mini Fillets	K+1	NL	79	16.7	4.3	840	350	542	525	20	0.67	0.56	0.61	-0.10	
E2	Mini Fillets	K+1	NL				900	370	528	490	20	0.76	0.80	0.78	0.04	
E3	Mini Fillets	K+1	NL				835	370	511	435	25	0.85	0.86	0.86	0.01	
E4	Mini Fillets	K+1	NL				820	390	501	465	20	0.84	0.72	0.78	-0.12	
E5	Mini Fillets	K+1	NL				865	390	524	490	25	0.80	0.71	0.76	-0.08	
Average							852	374	521.2	481	22	0.78	0.73	0.76	-0.05	0.76
S.D.							31	17	16	33	3	0.07	0.11			0.09
C.I.							39	21	20	42	3	0.09	0.14			0.07
Max							900	390	542	525	25	0.85	0.86			0.86
Min							820	350	501	435	20	0.67	0.56			0.56

Table 11f. Estimates of gas:meat ratios of five packs of thighs from Processor B

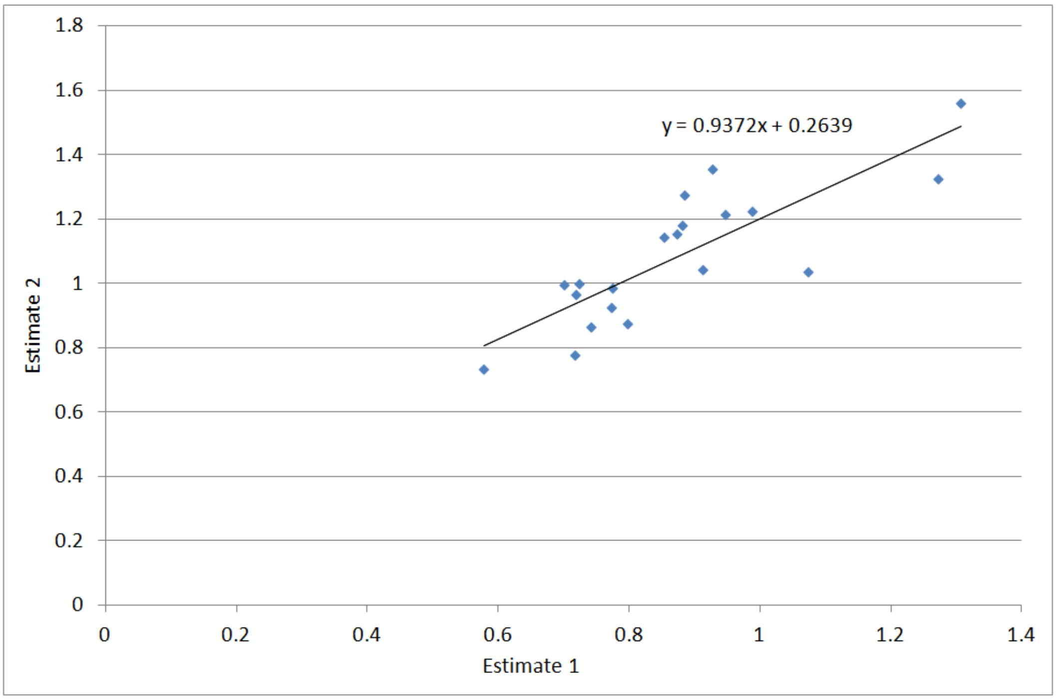
Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate 1 V_g/V_c	Estimate 2 $(V_{wp}-V_c-V_p)/V_c$	Average of Est1 and Est2	Difference Est2-Est1	Average based on 10 values
F1	Thighs	K+1	NL	74.9	14.9	10.2	2205	1275	915	900	25	1.42	1.42	1.42	0.01	
F2	Thighs	K+1	NL				2110	1230	925	885	25	1.39	1.36	1.37	-0.03	
F3	Thighs	K+1	NL				2130	1210	945	920	30	1.32	1.28	1.30	-0.03	
F4	Thighs	K+1	NL				2145	1300	836	790	30	1.65	1.68	1.66	0.03	
F5	Thighs	K+1	NL				2160	1320	881	835	30	1.58	1.55	1.57	-0.03	
Average							2150	1267	900.4	866	28	1.47	1.46	1.46	-0.01	1.46
S.D.							36	46	43	53	3	0.14	0.16			0.14
C.I.							45	58	53	66	3	0.17	0.20			0.10
Max							2205	1320	945	920	30	1.65	1.68			1.68
Min							2110	1210	836	790	25	1.32	1.28			1.28

Figure 3. Comparison of estimates of gas:meat ratios measured using two methods with one measuring the gas volume using a measuring cylinder (Estimate 1) and the other measuring the volume of individual components of the pack volume (Estimate 2)

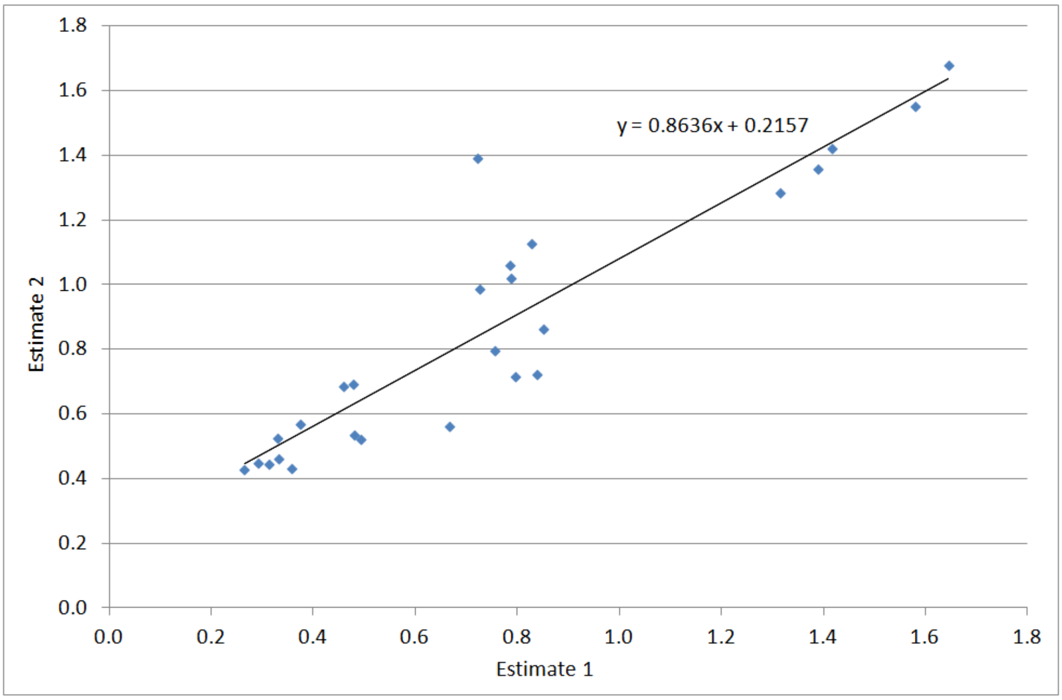
(a) Trial 1



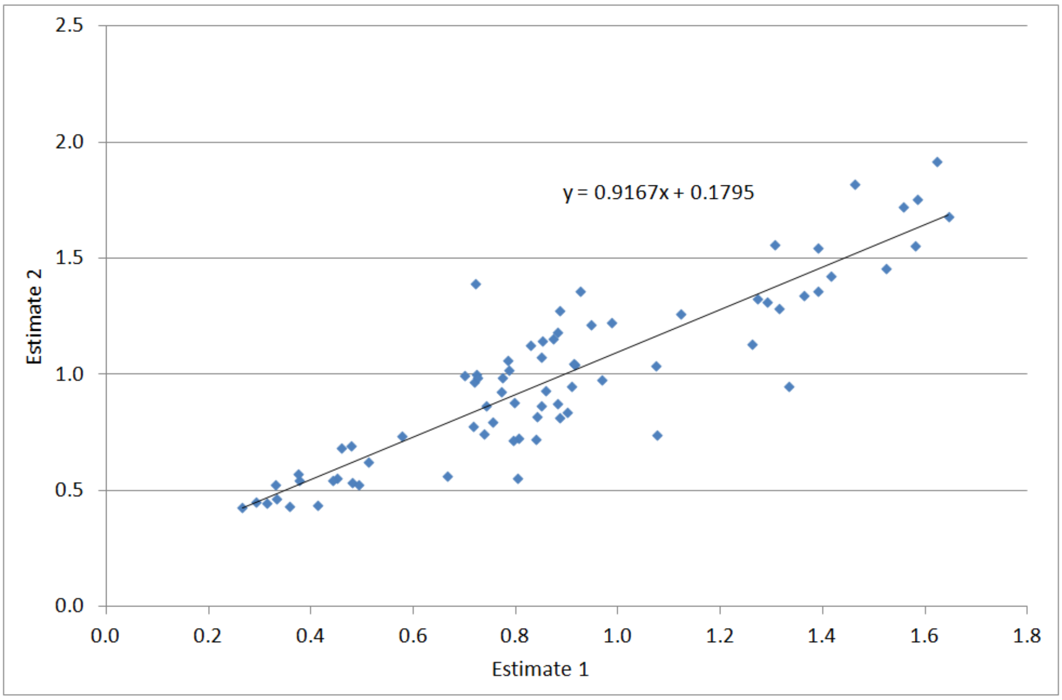
(b) Trial 2



(c) Trial 3



(d) All three trials



Figures 3a to 3d show the values of Estimate 2 plotted against Estimate 1 for each trial and for all of the trials combined. The slopes of the lines are close to 1 or just below that value. The offset is around 0.2 to 0.3 with Estimate 2 being a slightly higher estimate of the gas:meat ratio. Estimate 1 might be lower due to the difficulty of removing air from within the birds or from packs with dividers. Further analyses were based on Estimate 2 only and these values are summarised in Table 12 and Figure 4.

Except for one particular bird/pack format, all of the packs of whole bird had gas:meat ratios around 0.5. The exception was small birds in an uncommon gas mixture (16% O_2, 22% CO_2 and 62% N_2). As reported in Objective 1 of this project, most whole birds are packed in air or a mix of 80% O_2 and 20% CO_2. Packs of breasts had gas:meat ratios of around 0.8 and other products were in various gas:meat ratios.

5.4 Discussion and conclusions

The results from these trials suggested that the next part of this project, a large scale trial to examine the effects of current gas mixes on *Campylobacter* and others factors such as rancidity, should use a gas:meat ratio of 0.5.

The trials reported here were carried out on packs supplied by two processors. Five samples of a different form of pack, a semi-rigid dome, were obtained from a retail outlet. Information on the gas composition and gas:meat ratio for this pack format are shown in Table 13. Gas composition was 34% O_2, 15% CO_2, and 51% N_2, and the gas:meat ratio was 0.8. The gas composition at packing, based on information from processors, would have likely been 40% O_2, 10% CO_2, and 50% N_2. The use of this combination of pack, gas mixture, and gas:meat ratio was later discontinued by the processor and so was not considered further.

The results of the trials were presented and discussed at a meeting of the FSA/Industry Joint Working Group on *Campylobacter* on 7 November 2013. It was agreed that the industry and FSA needed to consider which days during storage samples should be tested during the large scale trial. In the proposal, it had been suggested that testing should be at 0, 4, 8, 12 and 16 days after kill and pack. The industry suggested that more frequent testing until only Day 10 might be more useful. The meeting also agreed that Objective 5, testing the variations of *Campylobacter* around the bird, should be carried out whilst the discussions on testing dates were considered. Consequently, the next section of this report considers Objective 5.

Table 12. Summary of gas:meat ratios and gas compositions measured during the three trials

(a) Trial 1

Product	Weight of meat, kg	O_2%	CO_2%	N_2%	Estimate 2 (Vwp-Vc-Vp)/Vc	Ratio S.D.
Thigh	1.033	27.2	16.2	56.6	0.86	0.08
Drumstic	0.571	23	24.1	52.90	1.70	0.25
Mini	0.264	84.5	13	2.50	1.36	0.16
Skin	0.389	86.9	10.4	2.70	0.76	0.14
Breast	0.648				0.96	0.10
Whole	1.447	80.3	14.3	5.40	0.54	0.07

(b) Trial 2

Product	Weight of meat, kg	O_2%	CO_2%	N_2%	Gas:Meat ratio	Ratio S.D.
Diced Breast	0.426	83.6	14.9	1.5	1.24	0.22
Drumstick	1.23	82.3	14.6	3.1	0.94	0.15
Breast	0.73	81.8	16	2.2	0.91	0.09
Leg	1.012	81.8	16.4	1.8	1.24	0.08

(c) Trial 3

Product	Weight of meat, kg	O_2%	CO_2%	N_2%	Gas:Meat ratio	Ratio S.D.
WB Large A	1.836	15.9	20	64.1	0.59	0.10
WB Small B	1.053	15.5	23.7	60.8	1.12	0.16
WB Large C	2.076	82.8	11.6	5.6	0.43	0.02
WB Small D	1.107	79.6	11.5	8.9	0.52	0.06
Mini Fillets	0.521	79	16.7	4.3	0.73	0.11
Thighs	0.900	74.9	14.9	10.2	1.46	0.16

Figure 4. Gas:meat ratios measured during the three trials shown with information on portion type, trial number, and oxygen concentration in the pack. Error bars are 95% confidence intervals

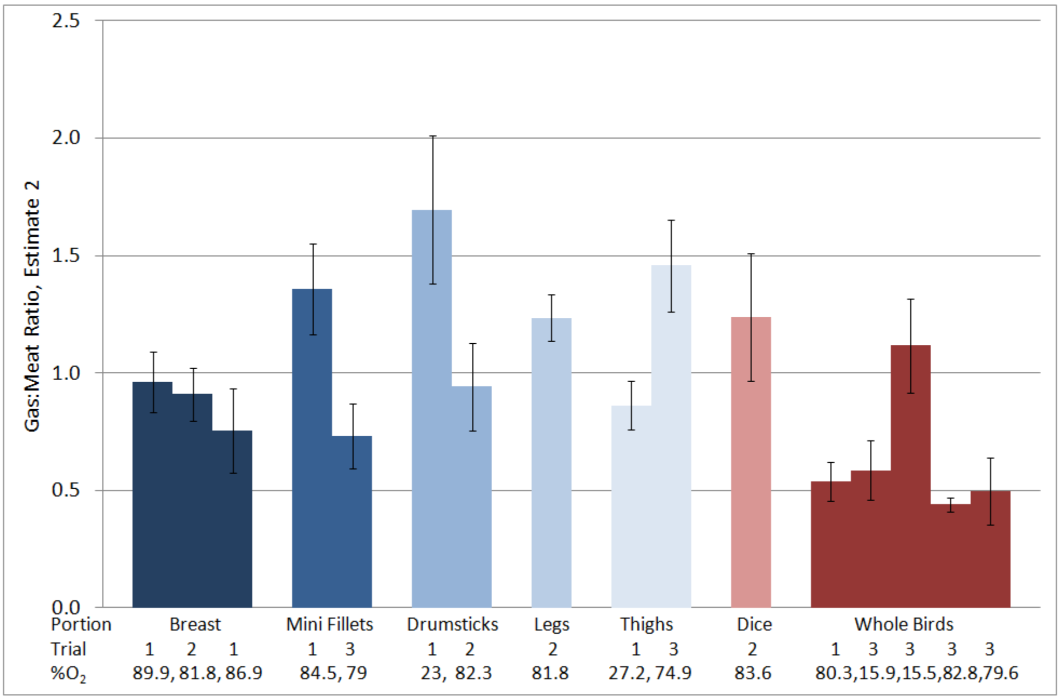


Table 13. Estimates ofgas:meat ratios of five packs of whole birds in semi-rigid dome packaging taken at retail

Pack No.	Format (thigh, leg etc)	Date of Packing	Weight on pack, kg	O_2%	CO_2%	N_2%	Volume of whole pack, ml (V_{wp})	Volume of gas in cylinder, ml (V_g)	Weight of meat, kg	Volume of unpacked chicken, ml (V_c)	Volume of packaging alone,ml (V_p)	Estimate2 (Vwp-Vc-Vp)/Vc
A1	Whole	NK, taken at retail	1.2	32.2	14.6	53.2	2660	[redacted]	1.537	1520	40	0.72
A2	Whole	NK, taken at retail	1.2	34.1	14.4	51.5	2520	[redacted]	1.398	1340	35	0.72
A3	Whole	NK, taken at retail	1.2	34.9	13.7	51.4	2480	[redacted]	1.316	1270	35	0.85
A4	Whole	NK, taken at retail	1.2	34.9	14.7	50.4	2530	[redacted]	1.49	1430	40	0.93
A5	Whole	NK, taken at retail	1.2	35.5	14.9	49.6	2570	[redacted]	1.47	1420	35	0.74
Average				34.3	14.5	51.2	2552	[redacted]	1.442	1396	37	0.79
S.D.				1.3	0.5	1.4	68	[redacted]	0.086	95	3	0.09
C.I.				1.6	0.6	1.7	85	[redacted]	0.107	118	3	0.11
Max				35.5	14.9	53.2	2660	[redacted]	1.537	1520	40	0.93
Min				32.2	13.7	49.6	2480	[redacted]	1.316	1270	35	0.72

6. TRIAL TO ASSESS THE VARIATION IN *CAMPYLOBACTER* COUNTS AROUND MODIFIED ATMOSPHERE PACKED (MAP) CARCASSES (TOP BREAST SKIN VERSUS BACK SKIN) (OBJECTIVE 5)

6.1 Introduction

The main objective of this FSA-funded project was to define the optimum gas mix to reduce the numbers of *Campylobacter* on chicken carcasses during storage. *Campylobacter* numbers are generally higher on the back skin on birds than on the breast skin and consequently greater reductions might be expected on the back. However, whole birds are packed with the back resting on the packing and the breast in contact with the gas mixture and this might suggest that reductions on the breast would be largest. The distribution of reductions in *Campylobacter* on whole birds is important because no process should aim to reduce campylobacters on just one part of the bird. The aim of this trial was to examine whether there are differences in the effects on *Campylobacter* on the breast and back skin.

6.2 Methods

A *Campylobacter* positive flock was identified by testing of boot swabs taken from farms. Samples had been sent to AFBI for *Campylobacter* testing using real time polymerase chain reaction (RT- PCR). Only one positive flock was identified for the processing at the plant in the week proposed for the trial. Those birds had an average live weight of 2.65 kg and killing on the day commenced at 11:55 and the first birds came out of the chiller at 14:18. Twenty five of the birds were packed on trays in a gas mix of 80% O_2 and 20% CO_2 in a low permeability film (Cryovac BDF film, Sealed Air, St Neots, UK). Another 25 birds were packed on trays in air in a high permeability film (Cryovac SES, Sealed Air, St Neots, UK). After packing, the birds were transported by chilled courier (4ºC) for 4.5 hours and then held for four days at 4ºC (Day K+4). Skin samples (25g) were removed from the breasts and backs of each bird and examined to determine the numbers of campylobacters. The detection limit for the quantitative method was 5 cfu /g.

The oxygen transfer rate (OTR) of two samples of each type of packaging was measured using a 8001 oxygen permeation analyzer manufactured by Systech Instruments, Thame, OX9 XA, UK in accordance with the manufacturer's instructions.

6.3 Results

The OTR of the low permeability (BDF) film was 10.2±0.3 cm^3/m^2/day and the OTR of the low barrier (SES) film was 11000±1170 cm^3/m^2/day, which was around a 1000-fold difference in OTR.

Table14 shows the numbers of *Campylobacter* on individual skin samples and the average log numbers. Student's t-tests showed no difference between the *Campylobacter* numbers on breast skin taken from birds packed in air or the high oxygen MAP ($p=0.361$) and no difference in *Campylobacter* numbers on back skins taken from birds packed in air or the high oxygen MAP ($p=0.799$). *Campylobacter* numbers on back skin samples were 0.4-log higher ($p=0.027$) than those on breast skin samples after the birds had been packed in the high oxygen mix. Similarly, *Campylobacter* numbers on back skin samples were 0.3-log higher than those on breast skin samples after the birds had been packed in air.

6.4 Conclusions and discussion

The higher *Campylobacter* numbers on the back skins was expected. The lack of any difference in *Campylobacter* numbers on samples packed in air or a high oxygen atmosphere was not expected. The samples came from only one flock. It was agreed with the FSA Project Officer to carry out a further trial of the effect of gas mixture but look at more flocks and just breast skin. Effectively, this would be part of Objective 4 but without testing of drip, colour and rancidity.

Table 14. *Campylobacter* numbers on breast and back skin samples taken from birds stored for 4 days in an 80% O_2 and 20% CO_2 mix in a low permeability film (10.2±0.3 $cm^3/m^2/day$) or in air in a high permeability film (11000±1170 $cm^3/m^2/day$) for 4 days

Bird No.	Sample No.	Air/MAP	Breast/Back	Count of confirmed Campylobacter per g	log(Campy)		Bird No.	Sample No.	Air/MAP	Breast/Back	Count of confirmed Campylobacter per g	log(Campy)
1	1	MAP	Breast	100.0	2.00		26	51	Air	Breast	110.0	2.04
2	3	MAP	Breast	185.0	2.27		27	53	Air	Breast	435.0	2.64
3	5	MAP	Breast	82.5	1.92		28	55	Air	Breast	172.5	2.24
4	7	MAP	Breast	145.0	2.16		29	57	Air	Breast	175.0	2.24
5	9	MAP	Breast	407.5	2.61		30	59	Air	Breast	275.0	2.44
6	11	MAP	Breast	82.5	1.92		31	61	Air	Breast	250.0	2.40
7	13	MAP	Breast	7.5	0.88		32	63	Air	Breast	25.0	1.40
8	15	MAP	Breast	80.0	1.90		33	65	Air	Breast	162.5	2.21
9	17	MAP	Breast	142.5	2.15		34	67	Air	Breast	22.5	1.35
10	19	MAP	Breast	<5	0.55		35	69	Air	Breast	97.5	1.99
11	21	MAP	Breast	152.5	2.18		36	71	Air	Breast	92.5	1.97
12	23	MAP	Breast	<5	0.55		37	73	Air	Breast	60.0	1.78
13	25	MAP	Breast	382.5	2.58		38	75	Air	Breast	80.0	1.90
14	27	MAP	Breast	42.5	1.63		39	77	Air	Breast	465.0	2.67
15	29	MAP	Breast	237.5	2.38		40	79	Air	Breast	80.0	1.90
16	31	MAP	Breast	47.5	1.68		41	81	Air	Breast	237.5	2.38
17	33	MAP	Breast	195.0	2.29		42	83	Air	Breast	70.0	1.85
18	35	MAP	Breast	180.0	2.26		43	85	Air	Breast	100.0	2.00
19	37	MAP	Breast	155.0	2.19		44	87	Air	Breast	552.5	2.74
20	39	MAP	Breast	210.0	2.32		45	89	Air	Breast	207.5	2.32
21	41	MAP	Breast	257.5	2.41		46	91	Air	Breast	125.0	2.10
22	43	MAP	Breast	187.5	2.27		47	93	Air	Breast	175.0	2.24
23	45	MAP	Breast	357.5	2.55		48	95	Air	Breast	92.5	1.97
24	47	MAP	Breast	427.5	2.63		49	97	Air	Breast	75.0	1.88
25	49	MAP	Breast	30.0	1.48		50	99	Air	Breast	187.5	2.27
Average					1.99		Average					2.12
S.D.					0.59		S.D.					0.35
C.I.					0.24		C.I.					0.14
N					25		N					25
n<5					2		n<5					0

Bird No.	Sample No.	Air/MAP	Breast/Back	Count of confirmed Campylobacter per g	log(Campy)		Bird No.	Sample No.	Air/MAP	Breast/Back	Count of confirmed Campylobacter per g	log(Campy)
1	2	MAP	Back	252.5	2.40		26	52	Air	Back	190.0	2.28
2	4	MAP	Back	1415.0	3.15		27	54	Air	Back	320.0	2.51
3	6	MAP	Back	95.0	1.98		28	56	Air	Back	485.0	2.69
4	8	MAP	Back	492.5	2.69		29	58	Air	Back	542.5	2.73
5	10	MAP	Back	742.5	2.87		30	60	Air	Back	487.5	2.69
6	12	MAP	Back	152.5	2.18		31	62	Air	Back	210.0	2.32
7	14	MAP	Back	330.0	2.52		32	64	Air	Back	140.0	2.15
8	16	MAP	Back	722.5	2.86		33	66	Air	Back	182.5	2.26
9	18	MAP	Back	57.5	1.76		34	68	Air	Back	112.5	2.05
10	20	MAP	Back	<5	0.55		35	70	Air	Back	210.0	2.32
11	22	MAP	Back	247.5	2.39		36	72	Air	Back	240.0	2.38
12	24	MAP	Back	<5	0.55		37	74	Air	Back	457.5	2.66
13	26	MAP	Back	212.5	2.33		38	76	Air	Back	222.5	2.35
14	28	MAP	Back	257.5	2.41		39	78	Air	Back	417.5	2.62
15	30	MAP	Back	215.0	2.33		40	80	Air	Back	75.0	1.88
16	32	MAP	Back	355.0	2.55		41	82	Air	Back	492.5	2.69
17	34	MAP	Back	270.0	2.43		42	84	Air	Back	262.5	2.42
18	36	MAP	Back	125.0	2.10		43	86	Air	Back	202.5	2.31
19	38	MAP	Back	650.0	2.81		44	88	Air	Back	497.5	2.70
20	40	MAP	Back	2010.0	3.30		45	90	Air	Back	170.0	2.23
21	42	MAP	Back	405.0	2.61		46	92	Air	Back	395.0	2.60
22	44	MAP	Back	560.0	2.75		47	94	Air	Back	242.5	2.38
23	46	MAP	Back	537.5	2.73		48	96	Air	Back	127.5	2.11
24	48	MAP	Back	750.0	2.88		49	98	Air	Back	407.5	2.61
25	50	MAP	Back	437.5	2.64		50	100	Air	Back	560.0	2.75
Average					2.39		Average					2.43
S.D.					0.65		S.D.					0.24
C.I.					0.27		C.I.					0.10
N					25		N					25
n<5					2		n<5					0

7. TRIAL TO ASSESS THE EFFECT OF STORAGE ON THE NUMBERS OF *CAMPYLOBACTER* ON CHICKEN CARCASSES STORED IN AN 80% O_2 MAP MIXTURE OR IN AIR (OBJECTIVE 4 WITHOUT ASSESSING EFFECT ON QUALITY)

7.1 Introduction

The previous trial found no evidence of a difference in *Campylobacter* numbers on the breast or back skin of birds stored in 80% O_2 in low permeability film and in air in high permeability film. Prior to carrying out very large scale tests on the effect of gas mixture, this trial was a repeat of the previous trial, described in Section 6, but focussing on breast skins and using birds from three batches. As in the previous trial, the birds were packed by a poultry processor.

7.2 Methods

The poultry processor identified three batches of birds, from three sheds on one farm, that were positive for *Campylobacter* based on testing of boot swabs samples by AFBI using PCR. Sixty birds, twenty from each batch, were to be packed in 80% O_2 in low permeability film and sixty birds, twenty from each batch, were to be packed in air in low permeability film. The processor generally packed birds in 80% O_2 in low permeability film (Cryovac BDF film, Sealed Air, St. Neots, UK) but did not use high permeability film (Cryovac SES film) on that site. For that reason, the following protocol was adopted. For each batch of birds, 20 birds were removed from the line at exit from the chiller and put into lined crates that there then placed back in the chiller until required for packing in the SES film. Twenty birds were removed from the production line after packing in the BDF film, the gas mix in each pack was measured using a Systech Instruments Gaspace Advance Model GS3/P (Systech Instruments Ltd Thame, Oxfordshire, OX9 3XA). This instrument measures the concentrations of oxygen and carbon dioxide and the balance is reported as nitrogen. The batches of birds left the chiller at 9:27, 9:57, and 12:55. After the third *Campylobacter* positive batch had been processed, a packing line was reconfigured to pack birds in air in SES film. The 60 birds that had been held in the chiller in crates were then packed on this line. All of the packed birds were weighed individually and put into lined crates. The birds were left in the factory dispatch area overnight and were then taken by chilled courier (4ºC) to the microbiology laboratory (4.5 hour journey). The birds were then held in a chiller for a further two days when microbiological sampling began (Day K+3).

Breast skin was removed from each bird and tested to determine numbers of *Campylobacter* to a limit of detection of 5 cfu/g (as in previous trials).

Five BDF films with trays and five SEE films with trays were weighed. The average weight for each type of film was subtracted from the weight of each packed bird, as measured at the factory, to obtain the weight of each bird.

7.3 Results

Tables 15a-15c show the gas compositions measured in the packs on Days 0 and K+3. Figure 5 shows the average concentration of oxygen in the packs from each of the batches on Day 0 was 76.4±1.9% (±95% C.I.), 76.4±1.0%, 78.1±1.2%. Some of the packs were noticeably stiffer (more inflated) than others and these packs tended, though not always, to have a higher oxygen concentration. One pack contained a gas mix with only 36% oxygen and that was replaced with a pack with a much higher oxygen concentration. The pack was found to have a leak.

The average concentration of oxygen in packs from each batch of birds dropped to the range 51-52% by Day K+3 and the confidence intervals on the averages rose to 10-13%. All of the measurements on packs containing air on Day 0 showed oxygen concentrations of 20.9% and this fell to 17% at Day K+3. The variation in oxygen concentration in these packs also increased from Day 0 to Day K+3. However, the variations in oxygen concentration in the air/SES packs were far less than those in the O_2/BDF packs.

Figure 6 shows the average, maximum and minimum oxygen concentrations on each day for each batch of birds and gas/pack format. This figure clearly shows the very large variations in gas composition in the BDF packs at Day K+3 and the smaller variations in the SES packs. The variations in the latter could arise from differences in the film permeability due to stretching during the formation of the pack and due to differences in the microbiological flora on the birds. Variations in the gas compositions in the BDF packs could come from the variations in the initial gas mix (relatively small), the permeability of the film, the microflora, and any leaks in the film. Very large leaks in the packs would result in very small carbon dioxide levels in the packs after three days. The results do show large variations in CO_2 concentrations but none of the packs have very low CO_2 concentrations. It would be interesting to know the variation in gas composition in packs at retail.

Unfortunately, for this trial, the breast skin samples showed so few campylobacters, and some showed no indication at all, that counting was not carried out. The flock was positive, as indicated by PCR testing of boot swabs, but the counts of *Campylobacter* were very low. For this reason no conclusions can be drawn on the effect of the gas mix on the numbers of *Campylobacter*. This was unfortunate as there was a good variation in gas mix in the packs at Day K+3 to enable a statistical analysis to test for a correlation between oxygen concentration and numbers of *Campylobacter*.

7.4 Conclusions

A repeat of this trial was agreed with the FSA Project Officer and this was to be carried out no earlier than May when *Campylobacter* numbers would be expected to be higher. A further trial was carried out in May 2014and another in July 2014 and those trials are described in the next sections of this report.

Table 15a. Bird weights and gas compositions measured in the packs for the first batch of birds on the day of kill and pack (Day 0) and on Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3		
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2						% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2
3	1	1	1.478	BDF	72.5	15.5	12.0	19.2	5.9	74.9	3	1	61	1.799	SES	20.9	0.5	78.6	16.1	4.8	79.1
3	1	2	1.462	BDF	75.4	16.7	7.9	19.1	7.5	73.4	3	1	62	1.816	SES	20.9	0.4	78.7	16.3	4.6	79.1
3	1	3	1.410	BDF	78.5	17.7	3.8	71.6	16.2	12.2	3	1	63	1.708	SES	20.9	0.5	78.6	17.2	4.3	78.5
3	1	4	1.598	BDF	66.9	15.0	18.1	20.1	5.4	74.5	3	1	64	1.587	SES	20.9	0.5	78.6	16.0	5.5	78.5
3	1	5	1.566	BDF	75.4	16.6	8.0	68.0	16.1	15.9	3	1	65	1.493	SES	20.9	0.4	78.7	16.6	4.0	79.4
3	1	6	1.397	BDF	78.6	17.5	3.9	36.2	15.3	48.5	3	1	66	1.888	SES	20.9	0.5	78.6	16.2	4.8	79.0
3	1	7	1.470	BDF	75.8	17.0	7.2	66.1	16.9	17.0	3	1	67	1.944	SES	20.9	0.5	78.6	16.7	4.1	79.2
3	1	8	1.455	BDF	69.0	14.9	16.1	20.5	5.2	74.3	3	1	68	1.776	SES	20.9	0.5	78.6	16.6	4.2	79.2
3	1	9	1.486	BDF	70.3	15.2	14.5	19.2	6.0	74.8	3	1	69	1.594	SES	20.9	0.5	78.6	16.2	5.1	78.7
3	1	10	1.306	BDF	80.5	14.8	4.7	67.3	14.7	18.0	3	1	70	1.676	SES	20.9	0.5	78.6	16.2	4.7	79.1
3	1	11	1.529	BDF	78.6	15.9	5.5	71.2	15.2	13.6	3	1	71	1.544	SES	20.9	0.5	78.6	17.1	4.0	78.9
3	1	12	1.583	BDF	81.3	16.3	2.4	78.4	17.0	4.6	3	1	72	1.717	SES	20.9	0.5	78.6	16.8	4.4	78.8
3	1	13	1.635	BDF	74.4	14.9	10.7	38.0	13.0	49.0	3	1	73	1.715	SES	20.9	0.5	78.6	17.3	4.3	78.4
3	1	14	1.463	BDF	79.6	16.1	4.3	71.1	17.0	11.9	3	1	74	1.640	SES	20.9	0.5	78.6	16.4	4.4	79.2
3	1	15	1.499	BDF	75.6	15.1	9.3	72.5	15.9	11.6	3	1	75	1.896	SES	20.9	0.5	78.6	16.6	4.6	78.8
3	1	16	1.481	BDF	79.7	15.8	4.5	36.9	12.2	50.9	3	1	76	1.663	SES	20.9	0.6	78.5	17.1	4.6	78.3
3	1	17	1.394	BDF	79.7	15.7	4.6	72.6	16.4	11.0	3	1	77	1.927	SES	20.9	0.6	78.5	17.6	4.3	78.1
3	1	18	1.445	BDF	80.0	16.1	3.9	67.5	16.0	16.5	3	1	78	1.388	SES	20.9	0.6	78.5	17.1	4.8	78.1
3	1	19	1.483	BDF	79.6	15.8	4.6	75.6	14.0	10.4	3	1	79	1.733	SES	20.9	0.6	78.5	17.0	4.7	78.3
3	1	20	1.511	BDF	77.5	15.5	7.0	25.9	9.6	64.5	3	1	80	1.820	SES	20.9	0.6	78.5	17.4	4.3	78.3
Mean			1.482		76.4	15.9	7.7	50.9	12.8	36.4				1.716		20.9	0.5	78.6	16.7	4.5	78.8
S.D.			0.077		4.1	0.9	4.5	23.8	4.4	27.8				0.148		0.0	0.1	0.1	0.5	0.4	0.4
C.I.			0.036		1.9	0.4	2.1	11.1	2.1	13.0				0.069		0.0	0.0	0.0	0.2	0.2	0.2
Max			1.635		81.3	17.7	18.1	78.4	17.0	74.9				1.944		20.9	0.6	78.7	17.6	5.5	79.4
Min			1.306		66.9	14.8	2.4	19.1	5.2	4.6				1.388		20.9	0.4	78.5	16.0	4.0	78.1
Spread			0.329		14.4	2.9	15.7	59.3	11.8	70.3				0.556		0.0	0.2	0.2	1.6	1.5	1.3
N			20		20	20	20	20	20	20				20		20	20	20	20	20	20

Table 15b. Bird weights and gas compositions measured in the packs for the second batch of birds on the day of kill and pack (Day 0) and on Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3		
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2						% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2
5	2	21	1.363	BDF	78.9	15.5	5.6	52.8	14.7	32.5	5	2	81	2.006	SES	20.9	0.5	78.6	18.2	3.9	77.9
5	2	22	1.437	BDF	76.8	16.9	6.3	20.9	9.2	69.9	5	2	82	1.918	SES	20.9	0.4	78.7	16.4	4.5	79.1
5	2	23	1.335	BDF	76.0	16.6	7.4	38.2	13.5	48.3	5	2	83	1.881	SES	20.9	0.4	78.7	16.2	4.6	79.2
5	2	24	1.450	BDF	76.5	16.9	6.6	46.4	14.3	39.3	5	2	84	1.824	SES	20.9	0.4	78.7	17.6	4.3	78.1
5	2	25	1.377	BDF	77.0	17.0	6.0	72.2	16.9	10.9	5	2	85	1.967	SES	20.9	0.4	78.7	16.7	4.4	78.9
5	2	26	1.388	BDF	77.1	16.7	6.2	38.2	12.0	49.8	5	2	86	1.456	SES	20.9	0.4	78.7	20.9	1.5	77.6
5	2	27	1.355	BDF	76.8	16.1	7.1	20.9	4.1	75.0	5	2	87	1.562	SES	20.9	0.4	78.7	16.9	4.3	78.8
5	2	28	1.331	BDF	76.1	16.4	7.5	77.2	16.9	5.9	5	2	88	1.459	SES	20.9	0.5	78.6	16.3	4.7	79.0
5	2	29	1.449	BDF	76.6	17.0	6.4	62.5	16.6	20.9	5	2	89	1.955	SES	20.9	0.5	78.6	16.7	4.3	79.0
5	2	30	1.487	BDF	77.4	17.2	5.4	35.8	10.2	54.0	5	2	90	1.523	SES	20.9	0.5	78.6	16.3	4.1	79.6
5	2	31	1.434	BDF	74.7	16.3	9.0	76.0	17.5	6.5	5	2	91	1.677	SES	20.9	0.6	78.5	16.4	4.3	79.3
5	2	32	1.453	BDF	76.4	16.6	7.0	76.1	16.8	7.1	5	2	92	1.693	SES	20.9	0.5	78.6	17.4	4.1	78.5
5	2	33	1.368	BDF	77.2	16.8	6.0	48.2	14.1	37.7	5	2	93	1.916	SES	20.9	0.6	78.5	16.3	4.5	79.2
5	2	34	1.406	BDF	75.9	16.6	7.5	28.6	10.9	60.5	5	2	94	1.635	SES	20.9	0.6	78.5	17.2	4.0	78.8
5	2	35	1.452	BDF	75.1	16.4	8.5	73.9	16.2	9.9	5	2	95	1.216	SES	20.9	0.6	78.5	16.5	4.1	79.4
5	2	36	1.452	BDF	75.0	15.2	9.8	56.5	14.2	29.3	5	2	96	1.745	SES	20.9	0.6	78.5	16.8	3.9	79.3
5	2	37	1.541	BDF	71.0	15.2	13.8	19.1	6.6	74.3	5	2	97	1.697	SES	20.9	0.5	78.6	16.8	4.2	79.0
5	2	38	1.434	BDF	72.4	16.3	11.3	20.9	8.4	70.7	5	2	98	1.721	SES	20.9	0.5	78.6	16.5	4.5	79.0
5	2	39	1.345	BDF	80.5	15.1	4.4	79.2	16.7	4.1	5	2	99	1.466	SES	20.9	0.5	78.6	16.6	4.5	78.9
5	2	40	1.311	BDF	80.5	16.1	3.4	77.4	17.3	5.3	5	2	100	1.642	SES	20.9	0.6	78.5	16.3	4.8	78.9
Mean			1.408		76.4	16.3	7.3	51.1	13.4	35.6				1.698		20.9	0.5	78.6	17.0	4.2	78.9
S.D.			0.060		2.2	0.6	2.4	22.2	3.9	25.9				0.210		0.0	0.1	0.1	1.1	0.7	0.5
C.I.			0.028		1.0	0.3	1.1	10.4	1.8	12.1				0.098		0.0	0.0	0.0	0.5	0.3	0.2
Max			1.541		80.5	17.2	13.8	79.2	17.5	75.0				2.006		20.9	0.6	78.7	20.9	4.8	79.6
Min			1.311		71.0	15.1	3.4	19.1	4.1	4.1				1.216		20.9	0.4	78.5	16.2	1.5	77.6
Spread			0.230		9.5	2.1	10.4	60.1	13.4	70.9				0.790		0.0	0.2	0.2	4.7	3.3	2.0
N			20		20	20	20	20	20	20				20		20	20	20	20	20	20

Table 15c. Bird weights and gas compositions measured in the packs for the third batch of birds on the day of kill and pack (Day 0) and on Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3		
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2						% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2
4	3	41	2.252	BDF	78.7	14.8	6.5	29.5	11.3	59.2	4	3	101	1.885	SES	20.9	0.4	78.7	19.2	3.7	77.1
4	3	42	2.058	BDF	79.1	16.2	4.7	21.6	9.0	69.4	4	3	102	1.824	SES	20.9	0.4	78.7	19.3	3.5	77.2
4	3	43	2.250	BDF	80.2	16.5	3.3	78.3	17.9	3.8	4	3	103	2.146	SES	20.9	0.5	78.6	18.2	4.2	77.6
4	3	44	2.062	BDF	80.1	16.4	3.5	20.9	8.3	70.8	4	3	104	1.867	SES	20.9	0.4	78.7	20.9	2.5	76.6
4	3	45	2.145	BDF	73.4	14.9	11.7	66.1	15.7	18.2	4	3	105	1.539	SES	20.9	0.5	78.6	16.4	4.9	78.7
4	3	46	2.080	BDF	73.0	12.4	14.6	70.6	13.2	16.2	4	3	106	1.924	SES	20.9	0.5	78.6	18.0	4.1	77.9
4	3	47	2.110	BDF	76.2	14.9	8.9	73.4	15.6	11.0	4	3	107	1.849	SES	20.9	0.6	78.5	16.8	4.5	78.7
4	3	48	2.115	BDF	78.0	15.5	6.5	75.4	16.1	8.5	4	3	108	1.479	SES	20.9	0.5	78.6	16.5	4.5	79.0
4	3	49	2.071	BDF	79.3	16.0	4.7	78.6	16.8	4.6	4	3	109	1.549	SES	20.9	0.6	78.5	17.6	4.4	78.0
4	3	50	2.066	BDF	80.1	16.0	3.9	78.8	17.0	4.2	4	3	110	1.934	SES	20.9	0.7	78.4	17.8	4.2	78.0
4	3	51	2.118	BDF	73.7	14.1	12.2	37.7	11.5	50.8	4	3	111	1.823	SES	20.9	0.7	78.4	17.0	4.3	78.7
4	3	52	2.096	BDF	79.5	15.6	4.9	19.5	5.6	74.9	4	3	112	2.004	SES	20.9	0.7	78.4	16.1	4.3	79.6
4	3	53	2.056	BDF	80.3	15.7	4.0	23.3	8.6	68.1	4	3	113	1.928	SES	20.9	0.7	78.4	19.9	3.2	76.9
4	3	54	2.250	BDF	77.7	14.9	7.4	20.9	8.9	70.2	4	3	114	1.633	SES	20.9	0.7	78.4	16.5	4.1	79.4
4	3	55	2.079	BDF	78.5	15.2	6.3	20.9	7.1	72.0	4	3	115	1.851	SES	20.9	0.8	78.3	17.1	4.1	78.8
4	3	56	2.169	BDF	79.9	15.3	4.8	20.1	7.8	72.1	4	3	116	1.677	SES	20.9	0.7	78.4	15.9	4.6	79.5
4	3	57	2.128	BDF	81.2	15.6	3.2	80.7	16.3	3.0	4	3	117	1.898	SES	20.9	0.8	78.3	17.5	4.2	78.3
4	3	58	2.109	BDF	78.0	14.9	7.1	75.0	15.9	9.1	4	3	118	2.015	SES	20.9	0.8	78.3	16.5	4.4	79.1
4	3	59	2.083	BDF	75.1	14.4	10.5	72.0	16.9	11.1	4	3	119	1.648	SES	20.9	0.8	78.3	15.9	4.7	79.4
4	3	60	2.030	BDF	79.9	15.2	4.9	77.0	17.9	5.1	4	3	120	1.377	SES	20.9	0.7	78.4	16.7	4.1	79.2
Mean			2.116		78.1	15.2	6.7	52.0	12.9	35.1				1.793		20.9	0.6	78.5	17.5	4.1	78.4
S.D.			0.067		2.5	0.9	3.3	26.6	4.2	30.7				0.200		0.0	0.1	0.1	1.4	0.5	0.9
C.I.			0.031		1.2	0.4	1.5	12.5	2.0	14.4				0.094		0.0	0.1	0.1	0.7	0.3	0.4
Max			2.252		81.2	16.5	14.6	80.7	17.9	74.9				2.146		20.9	0.8	78.7	20.9	4.9	79.6
Min			2.030		73.0	12.4	3.2	19.5	5.6	3.0				1.377		20.9	0.4	78.3	15.9	2.5	76.6
Spread			0.222		8.2	4.1	11.4	61.2	12.3	71.9				0.769		0.0	0.4	0.4	5.0	2.4	3.0
N			20		20	20	20	20	20	20				20		20	20	20	20	20	20

Figure 5. Average oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bards show 95% confidence intervals

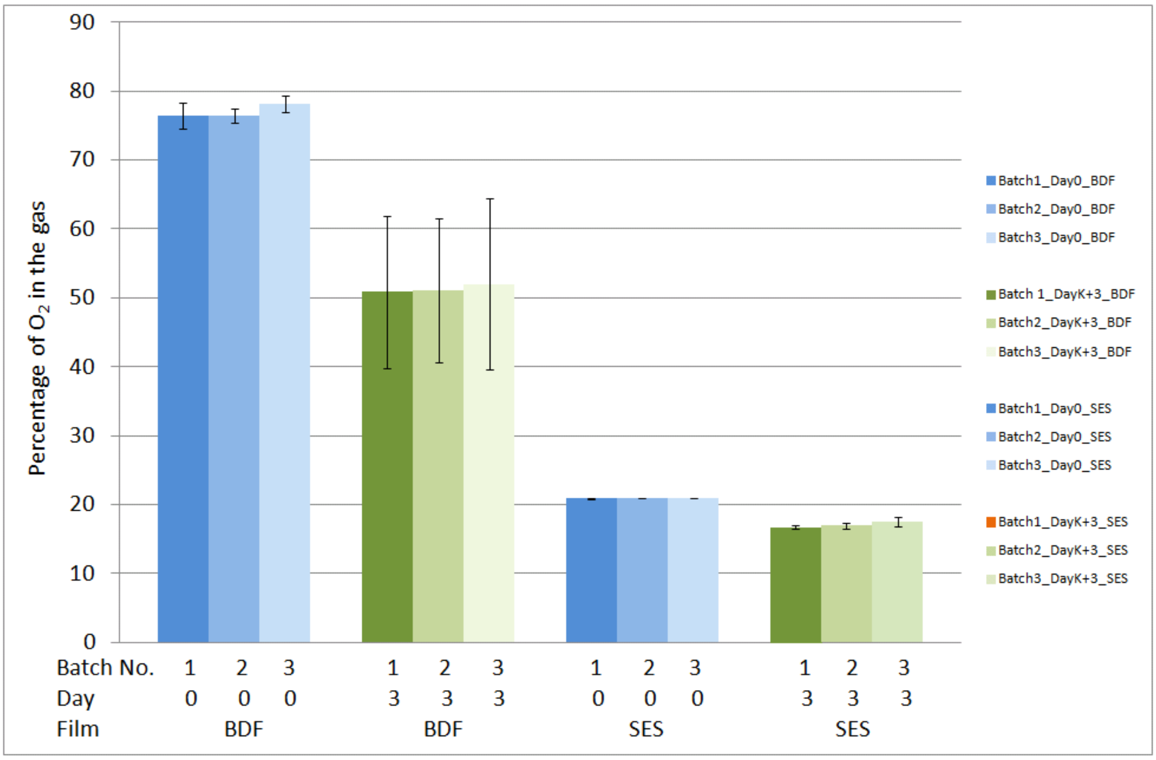
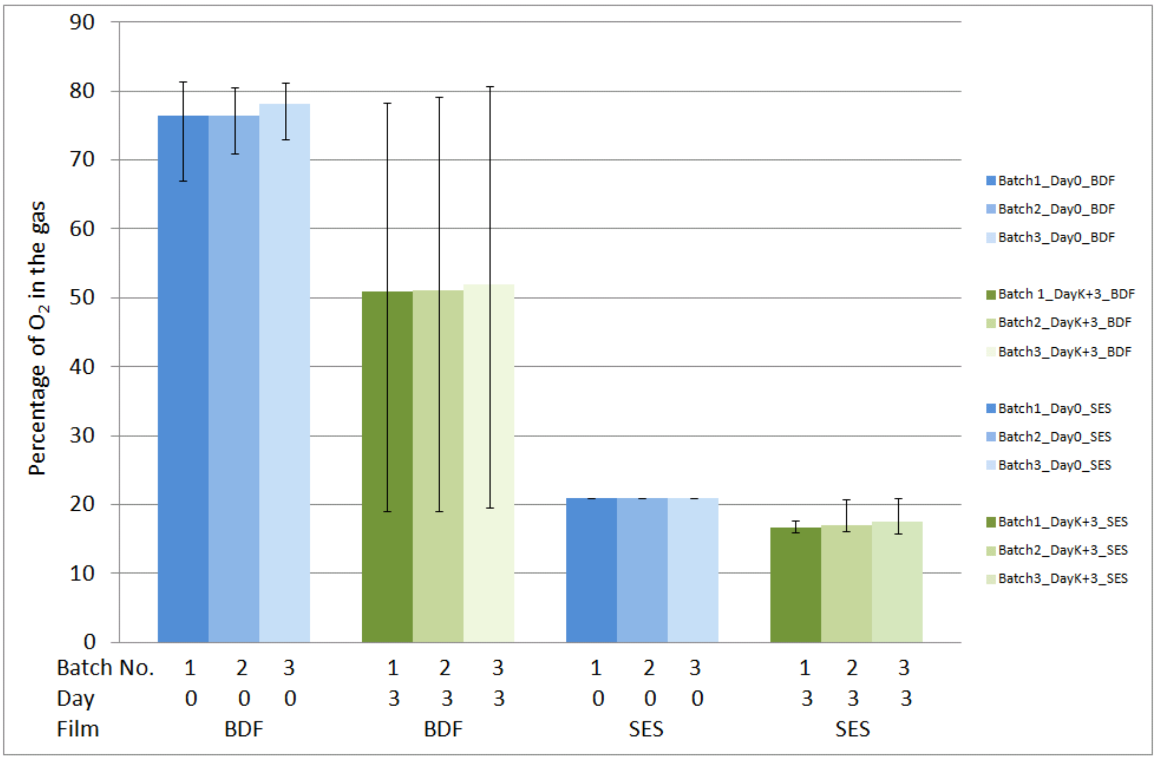


Figure 6. Average, minimum and maximum oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bars show the minimum and maximum values



8 TRIALTO ASSESS THE EFFECT OF STORAGE ON THE NUMBERS OF *CAMPYLOBACTER* ON CHICKEN CARCASSES STORED IN AN 80% O_2 MAP MIXTURE OR IN AIR (REPEAT OF OBJECTIVE 4 WITHOUT ASSESSING EFFECT ON QUALITY)

8.1 Introduction

This trial was a repeat of the previous one except that birds from two sheds were used.

8.2 Methods

The poultry processor identified four batches of birds, from four sheds on one farm, that were positive for *Campylobacter* based on testing of boot swabs samples by AFBI using PCR. However, on the day of the trial, only birds from two of the positive sheds were processed. Sixty birds, forty from one batch and twenty from the other, were packed in 80% O_2 in low permeability film and sixty birds, forty from one batch and twenty from the other, were to be packed in air in low permeability film. The processor generally packed birds in 80% O_2 in low permeability film (Cryovac BDF film, Sealed Air, St. Neots, UK) but did not use high permeability film (Cryovac SES film) on that site.

In the previous trial, birds for packing in air in the SES film were held in the chiller until the end of the day and then packed. In the current trial, birds were packed in SES film as soon as they left the chiller. The birds packed in BDF film were also packed immediately after chilling. The gas mix in each pack was measured immediately after packing using a Systech Instruments Gaspace Advance Model GS3/P (Systech Instruments Ltd Thame, Oxfordshire, OX9 3XA). This instrument measured the concentrations of oxygen and carbon dioxide and the balance was reported as nitrogen. The batches of birds left the chiller at 17:55 hrs (80 birds for testing), and 20:32 (40 birds for testing). All of the packed birds were weighed individually and put into lined crates. The birds were left in the factory dispatch area overnight and were then taken by chilled courier (4ºC) to the microbiology laboratory (6 hour journey). The birds were then held in a chiller for a further two days when the gas mixture was measured again and microbiological sampling was carried out (Day K+3). Breast skin was removed from each bird and tested to determine numbers of *Campylobacter* to a limit of detection of 5 cfu/g (as in previous trials).

8.3 Results

Tables 16a-16b show the gas compositions measured in the packs on Days 0 and K+3. Figure 7 shows the average concentration of oxygen in the packs and Figure 8 shows the averages along with the maximum and minimum concentrations.

The average concentration of oxygen in the BDF packs in Batch 1 fell from 75.7% down to 64.6% from Day 0 to Day K+3. The average concentration of oxygen in the BDF packs in Batch 2, fell from 69.1% down to 32.3% from Day 0 to Day K+3. Clearly, there were large differences in the oxygen concentration in the two batches.

All of the measurements on packs containing air on Day 0 showed oxygen concentrations of 20.9% and this fell to 18% at Day K+3. As in the previous trial, the variation in oxygen concentration in these packs also increased from Day 0 to Day K+3. However, the variations in oxygen concentration in the air/SES packs were far less than those in the O_2/BDF packs.

Figure 8 shows the very large variations in gas composition in the BDF packs at Day 0 and at Day K+3. The low oxygen concentration in some packs at Day 0 suggests that they were leaking.

Table 16 shows that the numbers of *Campylobacter* on the breast skin samples were very low and, on average, were less than10 cfu/g. For this reason, analysis of the data to test for an effect of the gas mix on numbers of *Campylobacter* was not justified.

8.4 Conclusions

A repeat of this trial was required. A different poultry site was used with the aim of sourcing flocks colonised with higher numbers of *Campylobacter*.

Table 16a. Bird weights and gas compositions measured in the packs for the first batch of birds on the day of kill and pack (Day 0) and on Day K+3. The table also shows numbers of *Campylobacter* on breast skin samples at Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas Compostion at Day 0			Gas composition at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
4	1	1	1.30	BDF	77.9	15.6	6.5	74	16.6	9.4	6	0.78
4	1	2	1.78	BDF	78.8	17	4.2	72.9	16.5	10.6	1	0.00
4	1	3	1.72	BDF	72.9	15.3	11.8	66	16.5	17.5	3	0.48
4	1	4	2.08	BDF	74.3	15.5	10.2	68.3	16.5	15.2	<1	-0.15
4	1	5	1.92	BDF	76	15.8	8.2	69.9	16.8	13.3	2	0.30
4	1	6	1.94	BDF	76.5	16	7.5	69.9	15.1	15	3	0.48
4	1	7	1.92	BDF	74	15.4	10.6	58.1	15.1	26.8	2	0.30
4	1	8	1.64	BDF	79.1	16.5	4.4	75	16.9	8.1	3	0.48
4	1	9	1.99	BDF	75.8	15.9	8.3	68.9	17.4	13.7	2	0.30
4	1	10	1.89	BDF	77.7	16	6.3	47.7	13.9	38.4	14	1.15
4	1	11	1.87	BDF	70.7	12.1	17.2	67.3	15.8	16.9	2	0.30
4	1	12	1.77	BDF	71.1	13.5	15.4	62.1	14.1	23.8	3	0.48
4	1	13	2.02	BDF	66.2	12.1	21.7	68.6	15.9	15.5	3	0.48
4	1	14	1.62	BDF	72.2	13.5	14.3	45	10.8	44.2	4	0.60
4	1	15	1.43	BDF	63.1	11.2	25.7	56.4	14.1	29.5	1	0.00
4	1	16	1.76	BDF	79.9	15.1	5	45.5	12.2	42.3	4	0.60
4	1	17	1.56	BDF	78.7	15	6.3	23	7.9	69.1	13	1.11
4	1	18	1.53	BDF	79.4	15.1	5.5	37	11.3	51.7	1	0.00
4	1	19	1.44	BDF	72.3	13.7	14	62.1	15.2	22.7	2	0.30
4	1	20	1.81	BDF	77.6	14.7	7.7	74.2	16	9.8	<1	-0.15
4	1	21	1.65	BDF	80.1	15	4.9	78.6	14.3	7.1	1	0.00
4	1	22	1.71	BDF	78.2	14.7	7.1	69.8	14.7	15.5	1	0.00

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas Compostion at Day 0			Gas composition at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
4	1	61	1.35	SES	20.9	0.4	78.7	18.5	4.1	77.4	2	0.30
4	1	62	1.74	SES	20.9	0.4	78.7	19.6	4.2	76.2	9	0.95
4	1	63	1.72	SES	20.9	0.3	78.8	17.9	4.5	77.6	1	0.00
4	1	64	1.68	SES	20.9	0.7	78.4	18.7	5.3	76	3	0.48
4	1	65	1.73	SES	20.9	0.8	78.3	16.2	4.9	78.9	2	0.30
4	1	66	1.82	SES	20.9	0.6	78.5	17.3	4.2	78.5	12	1.08
4	1	67	1.96	SES	20.9	0.9	78.2	17.6	4.3	78.1	3	0.48
4	1	68	1.41	SES	20.9	0.9	78.2	17.6	4.8	77.6	1	0.00
4	1	69	1.81	SES	20.9	0.9	78.2	16.9	5	78.1	1	0.00
4	1	70	1.27	SES	20.9	1	78.1	18.2	4.6	77.2	13	1.11
4	1	71	1.61	SES	20.9	0.9	78.2	19.2	3.9	76.9	14	1.15
4	1	72	1.36	SES	20.9	1	78.1	19.4	3.7	76.9	7	0.85
4	1	73	1.51	SES	20.9	1.3	77.8	17.7	5.5	76.8	1	0.00
4	1	74	1.45	SES	20.9	0.7	78.4	20.9	1.2	77.9	2	0.30
4	1	75	2.02	SES	20.9	0.6	78.5	17.6	4.5	77.9	1	0.00
4	1	76	2.05	SES	20.9	1.1	78	18.7	4.7	76.6	3	0.48
4	1	77	1.65	SES	20.9	1.1	78	20.9	2.3	76.8	4	0.60
4	1	78	1.84	SES	20.9	1.4	77.7	18.1	4.5	77.4	9	0.95
4	1	79	1.86	SES	20.9	1.1	78	20	3.4	76.6	2	0.30
4	1	80	1.43	SES	20.9	1.2	77.9	19.5	3.3	77.2	3	0.48
4	1	81	1.83	SES	20.9	1.3	77.8	18.9	3.8	77.3	7	0.85
4	1	82	2.1	SES	20.9	1.4	77.7	17.7	4.2	78.1	<1	-0.15

Table 16a Continued…..

4	1	23	1.65	BDF	80.6	15.2	4.2	77.5	16.1	6.4	4	0.60		4	1	83	1.66	SES	20.9	1.4	77.7	18.8	3.9	77.3	5	0.70
4	1	24	1.99	BDF	78.1	14.7	7.2	76.8	16.5	6.7	7	0.85		4	1	84	2.11	SES	20.9	1.3	77.8	20.9	1.4	77.7	1	0.00
4	1	25	1.59	BDF	77.6	14.6	7.8	74.3	16.1	9.6	<1	-0.15		4	1	85	1.74	SES	20.9	1.3	77.8	17.7	4.4	77.9	<1	-0.15
4	1	26	1.52	BDF	80.3	15	4.7	70.7	13.6	15.7	1	0.00		4	1	86	1.9	SES	20.9	1.2	77.9	17.6	4.4	78	<1	-0.15
4	1	27	1.88	BDF	80	15	5	79.7	15.3	5	3	0.48		4	1	87	1.68	SES	20.9	1.2	77.9	17.9	4.4	77.7	<1	-0.15
4	1	28	1.88	BDF	79.9	15	5.1	61.8	14.6	23.6	5	0.70		4	1	88	1.66	SES	20.9	1.1	78	17.5	4.5	78	2	0.30
4	1	29	2.23	BDF	78.5	14.7	6.8	76	16	8	1	0.00		4	1	89	1.65	SES	20.9	1.4	77.7	17.9	4.9	77.2	1	0.00
4	1	30	2.14	BDF	77.8	14.6	7.6	78	15.6	6.4	3	0.48		4	1	90	1.48	SES	20.9	1.3	77.8	18	4.1	77.9	9	0.95
4	1	31	2.27	BDF	80.5	14.8	4.7	79.7	14.6	5.7	2	0.30		4	1	91	1.97	SES	20.9	1.5	77.6	17.5	3.9	78.6	1	0.00
4	1	32	1.66	BDF	80.3	15	4.7	80	16.3	3.7	3	0.48		4	1	92	1.94	SES	20.9	1.2	77.9	17.8	4.4	77.8	6	0.78
4	1	33	1.47	BDF	80.6	15.3	4.1	79.5	17	3.5	14	1.15		4	1	93	2.08	SES	20.9	1.6	77.5	19.2	2.3	78.5	1	0.00
4	1	34	1.63	BDF	77.5	14.6	7.9	23	9.3	67.7	4	0.60		4	1	94	1.69	SES	20.9	1.4	77.5	17.1	4.1	78.8	2	0.30
4	1	35	2.06	BDF	79.7	14.4	5.9	77.2	14.9	7.9	4	0.60		4	1	95	1.55	SES	20.9	1.4	77.7	17.3	4.8	77.9	16	1.20
4	1	36	1.52	BDF	75.7	14.2	10.1	69.2	16.4	14.4	5	0.70		4	1	96	1.99	SES	20.9	1.6	77.5	19.3	3.8	76.9	16	1.20
4	1	37	1.98	BDF	71.5	12.8	15.7	63.3	15.6	21.1	2	0.30		4	1	97	1.92	SES	20.9	1.7	77.4	17.3	3.6	79.1	14	1.15
4	1	38	1.57	BDF	78.1	12.8	9.1	70.2	16.4	13.4	7	0.85		4	1	98	2.2	SES	20.9	1.6	77.5	18.7	3.6	77.7	18	1.26
4	1	39	1.46	BDF	76.3	13.9	9.8	68.1	15.7	16.2	3	0.48		4	1	99	2.08	SES	20.9	1.7	77.4	17.9	4.2	77.9	3	0.48
4	1	40	1.64	BDF	41.8	7	51.2	17.5	6.1	76.4	2	0.30		4	1	100	1.65	SES	20.9	1.5	77.6	20.9	1.6	77.5	2	0.30
Mean			1.76		75.7	14.5	9.9	64.6	14.7	20.7		0.41		Mean			1.75		20.9	1.1	78.0	18.4	4.0	77.6		0.47
S.D.			0.24		6.8	1.7	8.3	16.2	2.5	18.4		0.35		S.D.			0.24		0.0	0.4	0.4	1.2	1.0	0.7		0.46
C.I.			0.08		2.2	0.5	2.7	5.2	0.8	5.9		0.11		C.I.			0.08		0.0	0.1	0.1	0.4	0.3	0.2		0.15
Max			2.27		80.6	17.0	51.2	80.0	17.4	76.4		1.15		Max			2.20		20.9	1.7	78.8	20.9	5.5	79.1		1.26
Min			1.30		41.8	7.0	4.1	17.5	6.1	3.5		-0.15		Min			1.27		20.9	0.3	77.4	16.2	1.2	76.0		-0.15
Spread			0.98		38.8	10.0	47.1	62.5	11.3	72.9		1.30		Spread			0.93		0.0	1.4	1.4	4.7	4.3	3.1		1.41
N			40		40	40	40	40	40	40		40		N			40		40	40	40	40	40	40		40
n<1												3		n<1												4

Table 16b. Bird weights and gas compositions measured in the packs for the second batch of birds on the day of kill and pack (Day 0) and on Day K+3. The table also shows numbers of *Campylobacter* on breast skin samples at Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas Compostion at Day 0			Gas composition at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
6	2	41	2.05	BDF	73.7	14.2	12.1	23.2	8.5	68.3	3	0.48
6	2	42	2.08	BDF	67.4	13.5	19.1	20.9	3.2	75.9	42	1.62
6	2	43	1.85	BDF	51.1	9	39.9	20.9	2.8	76.3	2	0.30
6	2	44	2.28	BDF	69.9	13.5	16.6	20.9	3.2	75.9	9	0.95
6	2	45	1.86	BDF	74.5	15.1	10.4	19.3	6.4	74.3	4	0.60
6	2	46	1.74	BDF	76.8	15.7	7.5	20.6	7	72.4	5	0.70
6	2	47	1.82	BDF	79.6	16.6	3.8	59.1	14.2	26.7	1	0.00
6	2	48	1.91	BDF	77	15.8	7.2	19.1	6.6	74.3	31	1.49
6	2	49	1.69	BDF	62.6	11.7	25.7	20.9	7.8	71.3	6	0.78
6	2	50	1.62	BDF	74.5	15.2	10.3	69.5	15.3	15.2	3	0.48
6	2	51	1.81	BDF	52.8	9.3	37.9	20.9	0.5	78.6	3	0.48
6	2	52	1.81	BDF	79.6	15.7	4.7	19	5.2	75.8	2	0.30
6	2	53	1.83	BDF	52.3	9.3	38.5	18.8	6	75.2	1	0.00
6	2	54	2.14	BDF	71.5	13.3	15.2	19.1	7.4	73.5	3	0.48
6	2	55	1.93	BDF	79.3	15.5	5.2	73.9	15.4	10.7	5	0.70
6	2	56	1.82	BDF	77.6	15.5	6.9	68.4	16.8	14.8	2	0.30
6	2	57	1.91	BDF	54.2	9.1	36.7	19.3	7.9	72.8	7	0.85
6	2	58	1.87	BDF	67.8	11.9	20.3	19.2	7.7	73.1	7	0.85
6	2	59	1.85	BDF	58.5	9.9	31.6	20.9	2.1	77	1	0.00
6	2	60	1.71	BDF	80.6	15.5	3.9	72.6	15	12.4	12	1.08
Mean			1.88		69.1	13.3	17.7	32.3	8.0	59.7		0.62
S.D.			0.16		10.2	2.7	12.9	21.7	4.9	26.2		0.45
C.I.			2.09		2.1	2.1	2.1	2.1	2.1	2.1		2.09
Max			2.28		80.6	16.6	39.9	73.9	16.8	78.6		1.62
Min			1.62		51.1	9.0	3.8	18.8	0.5	10.7		0.00
Spread			0.66		29.5	7.6	36.1	55.1	16.3	67.9		1.62
N			20		20	20	20	20	20	20		20
n<1												0

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas Compostion at Day 0			Gas composition at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
6	2	101	1.97	SES	20.9	1.5	77.6	16.5	4.6	78.9	1	0.00
6	2	102	1.95	SES	20.9	0.7	78.4	16.8	4.6	78.6	31	1.49
6	2	103	2.28	SES	20.9	0.6	78.5	16.1	4.8	79.1	1	0.00
6	2	104	1.67	SES	20.9	0.5	78.6	17.4	3.8	78.8	20	1.30
6	2	105	1.88	SES	20.9	0.6	78.5	16.4	4.8	78.8	6	0.78
6	2	106	2.18	SES	20.9	0.6	78.5	18.1	4.5	77.4	6	0.78
6	2	107	1.76	SES	20.9	0.6	78.5	17.7	4.9	77.4	<1	-0.15
6	2	108	2.23	SES	20.9	0.7	78.4	18.7	4.2	77.1	4	0.60
6	2	109	1.85	SES	20.9	0.7	78.4	18.4	4.2	77.4	<1	-0.15
6	2	110	1.86	SES	20.9	0.6	78.5	17.7	4.5	77.8	<1	-0.15
6	2	111	1.77	SES	20.9	0.7	78.4	17.9	4.2	77.9	13	1.11
6	2	112	1.85	SES	20.9	0.6	78.5	18.7	4.1	77.2	5	0.70
6	2	113	2.27	SES	20.9	0.9	78.2	18.1	4.5	77.4	5	0.70
6	2	114	1.69	SES	20.9	0.7	78.4	17.4	4.3	78.3	1	0.00
6	2	115	1.73	SES	20.9	0.7	78.4	17.2	4.7	78.1	18	1.26
6	2	116	1.85	SES	20.9	0.7	78.4	18.2	4.3	77.5	38	1.58
6	2	117	2.23	SES	20.9	0.8	78.3	17.3	5.3	77.4	1	0.00
6	2	118	1.99	SES	20.9	0.8	78.3	17.8	5.2	77	5	0.70
6	2	119	1.75	SES	20.9	0.9	78.2	18.9	3.8	77.3	9	0.95
6	2	120	2.26	SES	20.9	0.9	78.2	17.9	4.4	77.7	13	1.11
Mean			1.95		20.9	0.7	78.4	17.7	4.5	77.9		0.63
S.D.			0.21		0.0	0.2	0.2	0.8	0.4	0.7		0.59
C.I.			2.09		2.1	2.1	2.1	2.1	2.1	2.1		2.09
Max			2.28		20.9	1.5	78.6	18.9	5.3	79.1		1.58
Min			1.67		20.9	0.5	77.6	16.1	3.8	77.0		-0.15
Spread			0.61		0.0	1.0	1.0	2.8	1.5	2.1		1.73
N			20		20	20	20	20	20	20		20
n<1												3

Figure 7. Average oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bars show 95% confidence intervals

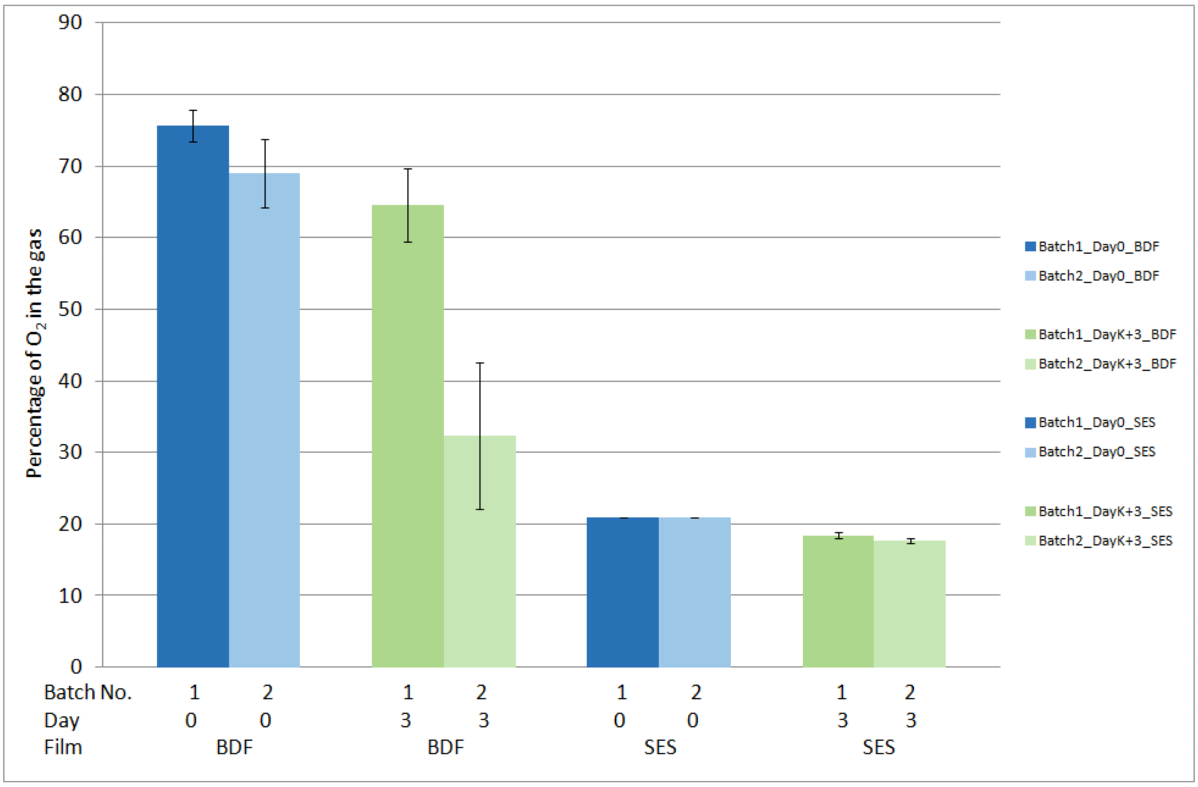
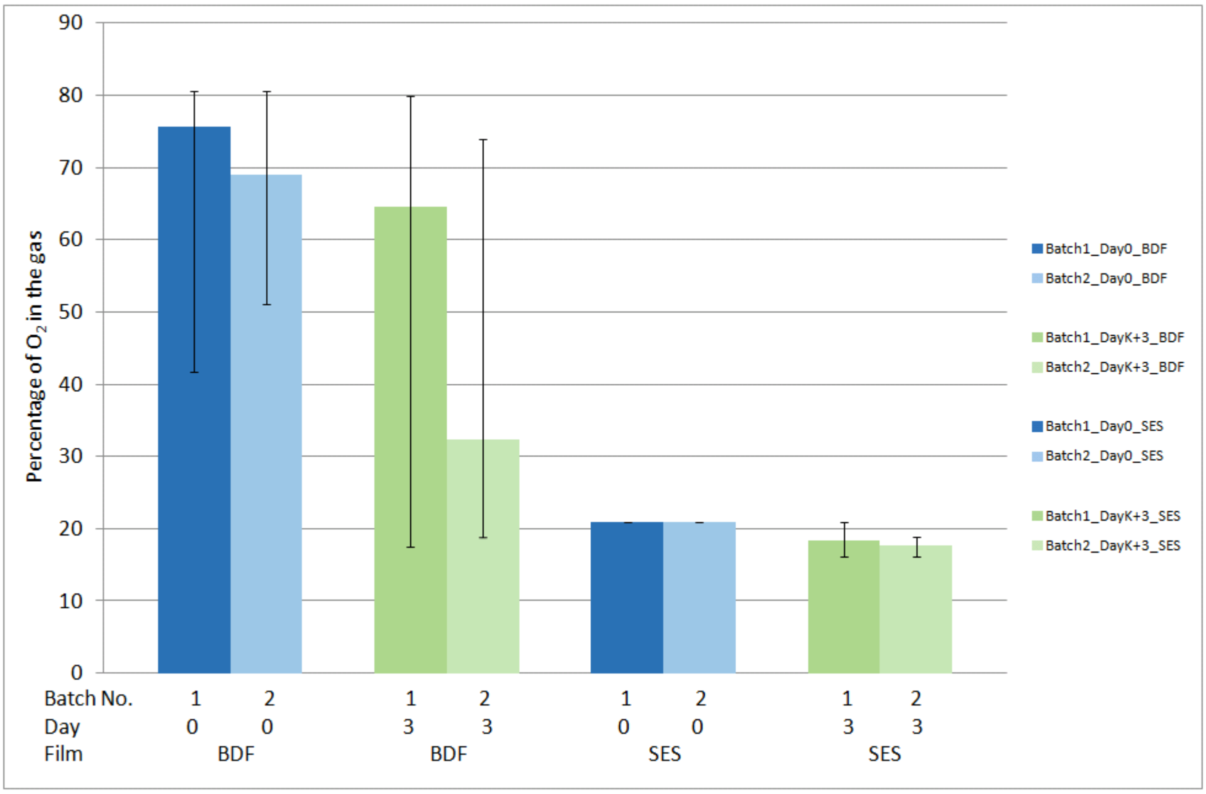


Figure 8. Average, minimum and maximum oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bars show the minimum and maximum values



9. TRIAL TO ASSESS THE EFFECT OF STORAGE ON THE NUMBERS OF *CAMPYLOBACTER* ON CHICKEN CARCASSES STORED IN AN 80% O_2 MAP MIXTURE OR IN AIR (FURTHER REPEAT OF OBJECTIVE 4 WITHOUT ASSESSING EFFECT ON QUALITY)

9.1 Introduction

Unfortunately, in much of the previous work, the samples showed so few campylobacters, and some showed no indication at all, that no conclusion could be drawn as to the effect of modified atmosphere packing on the numbers of *Campylobacter*. The trial reported here was a repeat of the earlier trial procedures.

9.2 Methods

The poultry processor identified four batches of birds, from two sheds on two farms, that were positive for *Campylobacter* based on testing of boot swabs samples by AFBI using PCR. On the day of the trial, trials were carried out with three batches of birds. The first batch came from two sheds from one farm, the next batch came from two sheds from another farm, and the third batch came from one of the sheds from the first farm. For each batch, twenty birds were packed in a high O_2 gas mixture in low permeability film and 20 others were to be packed in air in high permeability film. Packing occurred after grading and chilling. The target concentration of oxygen in the gas mixture was 70%. The low permeability film was Cryovac BDF film (Sealed Air, St. Neots, UK) and the high permeability film was Cryovac SES film.

The gas mix in each pack was measured immediately after packing using a Systech Instruments Gaspace Advance Model GS3/P (Systech Instruments Ltd Thame, Oxfordshire, OX9 3XA). This instrument measured the concentrations of oxygen and carbon dioxide and the balance was reported as nitrogen. All of the packed birds were weighed individually and put into lined crates. The birds were left in the factory dispatch area overnight and were then taken by chilled courier (4ºC) to the microbiology laboratory (6 hour journey). The birds were then held in a chiller for a further two days when the gas mixture was measured again and microbiological sampling was carried out (Day K+3). Breast skin was removed from each bird and tested to determine numbers of *Campylobacter* to a limit of detection of 2.5 cfu/g.

9.3 Results

Tables 17a-17c show the gas compositions measured in the packs on Days 0 and K+3. In five tests, the microaerophilic chambers used in the *Campylobacter* testing did not seal correctly and those tests have been ignored. Those five tests could not be repeated as all of the skin samples had been used. Figure 9 shows the average concentration of oxygen in the packs and Figure 10 shows the averages along with the maximum and minimum concentrations.

For the three batches, the average concentrations of oxygen in the BDF packs at Day 0 were 71, 72, and 72%. At Day K+3, the average concentrations were 58, 43 and 53%. The average concentrations of oxygen in the SES packs were between 20 and 21% at Day 0 and at 15% at Day K+3.

As in previous tests, very large variations in gas composition in the BDF packs were found at Day K+3 (Figure 10). Some packs retained very high oxygen concentrations whilst others had concentrations close to that of air.

Tables 17a-17c show the numbers of *Campylobacter* on the breast skin samples. Although, the numbers are quite low only a few of the samples showed numbers below the level of detection and a statistical comparison of the numbers of *Campylobacter* on birds packed in oxygen or air is valid. The average numbers of *Campylobacter* taken from birds packed in oxygen were 1.45 (C.I.=±0.37), 1.15 (±0.33), and 1.35 (±0.19) log cfu/g for batches 1, 2 and 3. The average numbers of *Campylobacter* taken from birds from the same batches but packed in air were 1.58 (±0.25), 1.07 (±0.21), and 1.34 (±0.33) log cfu/g. The results show no evidence of an effect of the concentration of oxygen on the numbers of *Campylobacter* after 3 days of storage.

9.4 Conclusions

Two trials have resulted in sufficient numbers of *Campylobacter* to test for an effect of the oxygen concentration in the retail packs. Both tests have found no evidence of an effect of the oxygen concentration on the numbers of *Campylobacter* on the chicken skin after 3 days of storage.

Table 17a. Bird weights and gas compositions measured in the packs for the first batch of birds on the day of kill and pack (Day 0) and on Day K+3. The table also shows numbers of *Campylobacter* on breast skin samples at Day K+3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
6&9	1	1	1.528	BDF	71.6	21.0	7.4	72.5	18.7	8.8	75.0	1.88
6&9	1	2	1.572	BDF	73.8	18.4	7.8	64.0	17.1	18.9	27.5	1.44
6&9	1	3	1.694	BDF	64.0	17.2	18.8	68.6	18.4	13.0	25.0	1.40
6&9	1	4	1.742	BDF	71.1	20.5	8.4	70.2	19.5	10.3	27.5	1.44
6&9	1	5	1.504	BDF	72.0	21.8	6.2	69.1	20.7	10.2	<2.5	0.25
6&9	1	6	1.686	BDF	75.4	19.6	5.0	70.6	19.0	10.4	5.0	0.70
6&9	1	7	1.490	BDF	69.6	19.6	10.8	28.4	15.4	56.2	72.5	1.86
6&9	1	8	1.618	BDF	75.0	19.5	5.5	52.5	17.6	29.9	12.5	1.10
6&9	1	9	1.542	BDF	66.4	19.5	14.1	52.8	18.0	29.2	22.5	1.35
6&9	1	10	1.448	BDF	74.0	18.7	7.3	72.1	19.0	8.9	15.0	1.18
6&9	1	11	1.684	BDF	73.0	19.6	7.4	61.5	19.3	19.2	135.0	2.13
6&9	1	12	1.534	BDF	76.1	19.7	4.2	73.9	19.6	6.5	580.0	2.76
6&9	1	13	1.532	BDF	66.0	18.1	15.9	20.9	10.2	68.9	50.0	1.70
6&9	1	14	1.754	BDF	71.0	18.6	10.4	28.4	12.3	59.3	17.5	1.24
6&9	1	15	1.702	BDF	70.5	19.2	10.3	18.2	9.4	72.4	155.0	2.19
6&9	1	16	1.588	BDF	75.8	19.3	4.9	72.7	19.5	7.8	12.5	1.10
6&9	1	17	1.722	BDF	62.4	20.7	16.9	71.9	17.7	10.4	<2.5	0.25
6&9	1	18	1.556	BDF	64.6	21.0	14.4	62.2	19.4	18.4	<2.5	0.25
6&9	1	19	1.725	BDF	71.9	22.3	5.8	59.1	21.2	19.7	40.0	1.60
6&9	1	20	1.540	BDF	72.5	22.5	5.0	69.4	21.2	9.4	1750.0	3.24
Mean			1.608		70.8	19.8	9.3	58.0	17.7	24.4		1.45
S.D.			0.096		4.1	1.4	4.5	18.6	3.4	21.7		0.78
C.I.			0.045		1.9	0.7	2.1	8.7	1.6	10.1		0.37
Max			1.754		76.1	22.5	18.8	73.9	21.2	72.4		3.24
Min			1.448		62.4	17.2	4.2	18.2	9.4	6.5		0.25
Spread			0.306		13.7	5.3	14.6	55.7	11.8	65.9		3.00
N			20		20	20	20	20	20	20		20
n<2.5												3

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
6&9	1	61	1.684	SES	20.9	0.6	78.5	14.6	5.1	80.3	35.0	1.54
6&9	1	62	1.700	SES	20.9	0.6	78.5	15.9	4.4	79.7	30.0	1.48
6&9	1	63	1.644	SES	20.9	0.6	78.5	16.3	4.4	79.3	7.5	0.88
6&9	1	64	1.644	SES	20.9	0.7	78.4	14.2	4.8	81.0	37.5	1.57
6&9	1	65	1.610	SES	20.9	0.7	78.4	14.5	4.8	80.7	35.0	1.54
6&9	1	66	1.742	SES	20.9	0.8	78.3	14.6	5.1	80.3	70.0	1.85
6&9	1	67	1.734	SES	20.9	0.8	78.3	16.8	4.7	78.5	5.0	0.70
6&9	1	68	1.562	SES	20.9	0.9	78.2	14.5	5.6	79.9	57.5	1.76
6&9	1	69	1.726	SES	20.9	0.8	78.5	15.6	4.7	79.7	151.7	2.18
6&9	1	70	1.744	SES	20.9	0.8	78.3	15.2	1.2	83.6	482.5	2.68
6&9	1	71	1.518	SES	20.9	0.8	78.3	14.6	4.9	80.5	285.0	2.45
6&9	1	72	1.586	SES	20.9	0.9	78.2	14.8	4.9	80.3	82.5	1.92
6&9	1	73	1.534	SES	20.9	0.9	78.2	14.6	5.0	80.4	47.5	1.68
6&9	1	74	1.702	SES	20.9	1.0	78.1	15.6	4.6	79.8	10.0	1.00
6&9	1	75	1.622	SES	20.9	1.0	78.1	15.2	5.0	79.8	7.5	0.88
6&9	1	76	1.420	SES	20.9	1.0	78.1	14.7	5.1	80.2	25.0	1.40
6&9	1	77	1.762	SES	20.9	1.0	78.1	16.2	4.6	79.2	12.5	1.10
6&9	1	78	1.696	SES	20.9	0.9	78.2	14.8	4.6	80.6	105.0	2.02
6&9	1	79	1.426	SES	20.9	1.0	78.1	15.8	4.7	79.5	60.0	1.78
6&9	1	80	1.720	SES	20.9	1.0	78.1	14.3	4.9	80.8	15.0	1.18
Mean			1.639		20.9	0.8	78.3	15.1	4.7	80.2		1.58
S.D.			0.103		0.0	0.1	0.2	0.7	0.9	1.0		0.53
C.I.			0.048		0.0	0.1	0.1	0.4	0.4	0.5		0.25
Max			1.762		20.9	1.0	78.5	16.8	5.6	83.6		2.68
Min			1.420		20.9	0.6	78.1	14.2	1.2	78.5		0.70
Spread			0.342		0.0	0.4	0.4	2.6	4.4	5.1		1.98
N			20		20	20	20	20	20	20		20
n<2.5												0

Table 17b. Bird weights and gas compositions measured in the packs for the second batch of birds on the day of kill and pack (Day 0) and on Day K+3. The table also shows numbers of *Campylobacter* on breast skin samples at Day K+3. BNS=Microaerophilic box not sealed correctly

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)	Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2								% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
4&3	2	21	1.586	BDF	74.8	19.9	5.3	17.6	7.8	74.6	67.5	1.83	4&3	2	81	1.498	SES	18.2	0.7	81.1	14.0	4.7	81.3	5.0	0.70
4&3	2	22	1.752	BDF	73.2	19.7	7.1	31.1	13.0	55.9	5.0	0.70	4&3	2	82	1.726	SES	18.9	0.7	80.4	14.6	4.6	80.8	10.0	1.00
4&3	2	23	1.712	BDF	67.0	18.6	14.4	37.9	17.0	45.1	1032.5	3.01	4&3	2	83	1.674	SES	19.0	0.7	80.3	14.9	4.6	80.5	100.0	2.00
4&3	2	24	1.646	BDF	74.4	19.6	6.0	69.3	19.9	10.8	2.5	0.40	4&3	2	84	1.562	SES	20.6	0.8	78.6	16.3	4.4	79.3	15.0	1.18
4&3	2	25	1.712	BDF	74.6	19.6	5.8	47.7	16.8	35.5	5.0	0.70	4&3	2	85	1.658	SES	20.3	0.8	78.9	15.9	4.7	79.4	5.0	0.70
4&3	2	26	1.626	BDF	75.4	19.4	5.2	27.8	13.2	59.0	10.0	1.00	4&3	2	86	1.580	SES	20.9	0.8	78.3	14.8	4.9	80.3	2.5	0.40
4&3	2	27	1.812	BDF	74.3	19.4	8.3	20.9	9.6	69.5	BNS		4&3	2	87	1.438	SES	20.9	0.6	78.5	13.3	5.6	81.1	25.0	1.40
4&3	2	28	1.748	BDF	74.1	19.6	6.3	20.6	10.7	68.7	20.0	1.30	4&3	2	88	1.570	SES	19.9	1.0	79.1	15.5	4.9	79.6	5.0	0.70
4&3	2	29	1.594	BDF	69.1	19.4	11.5	23.8	11.1	65.1	12.5	1.10	4&3	2	89	1.668	SES	20.0	0.9	79.1	14.8	4.6	80.6	50.0	1.70
4&3	2	30	1.586	BDF	72.9	19.7	7.4	30.3	14.9	54.8	27.5	1.44	4&3	2	90	1.682	SES	20.9	0.9	78.2	14.5	4.9	80.6	17.5	1.24
4&3	2	31	1.758	BDF	61.4	19.4	19.2	67.4	18.0	14.6	2.5	0.40	4&3	2	91	1.622	SES	20.9	1.0	78.1	14.8	5.1	80.1	50.0	1.70
4&3	2	32	1.712	BDF	72.6	19.2	8.2	45.0	17.8	37.2	5.0	0.70	4&3	2	92	1.588	SES	20.9	1.0	78.1	16.1	4.8	79.1	5.0	0.70
4&3	2	33	1.622	BDF	66.6	19.5	13.9	70.0	19.2	10.8	2.5	0.40	4&3	2	93	1.770	SES	20.5	1.1	78.4	14.6	4.9	80.5	10.0	1.00
4&3	2	34	1.688	BDF	72.7	21.2	6.1	39.2	18.0	42.8	17.5	1.24	4&3	2	94	1.590	SES	20.9	0.9	78.2	14.5	4.5	81.0	5.0	0.70
4&3	2	35	1.554	BDF	74.4	21.3	4.3	56.7	19.8	23.5	5.0	0.70	4&3	2	95	1.512	SES	18.2	1.1	80.7	14.7	4.8	80.5	22.5	1.35
4&3	2	36	1.594	BDF	72.6	21.6	5.8	28.9	14.7	56.4	<2.5	0.25	4&3	2	96	1.654	SES	20.8	1.0	78.2	14.7	4.8	80.5	7.5	0.88
4&3	2	37	1.752	BDF	74.5	21.5	4.0	50.6	19.1	30.3	122.5	2.09	4&3	2	97	1.446	SES	17.6	1.1	81.3	15.2	4.5	80.3	10.0	1.00
4&3	2	38	1.614	BDF	75.2	19.7	5.1	65.9	19.4	14.7	67.5	1.83	4&3	2	98	1.606	SES	18.8	1.3	79.9	15.8	4.7	79.5	52.5	1.72
4&3	2	39	1.568	BDF	75.1	21.1	3.8	69.4	19.1	11.5	52.5	1.72	4&3	2	99	1.548	SES	20.9	0.9	78.2	15.2	4.4	80.4	2.5	0.40
4&3	2	40	1.650	BDF	67.6	20.4	12.0	32.7	15.6	51.7	10.0	1.00	4&3	2	100	1.454	SES	20.9	0.9	78.2	17.7	3.8	78.5	10.0	1.00
Mean			1.664		72.1	20.0	8.0	42.6	15.7	41.6		1.15				1.592		20.0	0.9	79.1	15.1	4.7	80.2		1.07
S.D.			0.076		3.8	0.9	4.1	18.3	3.7	21.7		0.71				0.092		1.1	0.2	1.1	0.9	0.3	0.7		0.46
C.I.			0.036		1.8	0.4	1.9	8.6	1.7	10.2		0.33				0.043		0.5	0.1	0.5	0.4	0.2	0.3		0.21
Max			1.812		75.4	21.6	19.2	70.0	19.9	74.6		3.01				1.770		20.9	1.3	81.3	17.7	5.6	81.3		2.00
Min			1.554		61.4	18.6	3.8	17.6	7.8	10.8		0.25				1.438		17.6	0.6	78.1	13.3	3.8	78.5		0.40
Spread			0.258		14.0	3.0	15.4	52.4	12.1	63.8		2.77				0.332		3.3	0.7	3.2	4.4	1.8	2.8		1.60
N			20		20	20	20	20	20	20		19				20		20	20	20	20	20	20		20
n<2.5												1													0

Table 17c. Bird weights and gas compositions measured in the packs for the third batch of birds on the day of kill and pack (Day 0) and on Day K+3. The table also shows numbers of *Campylobacter* on breast skin samples at Day K+3. BNS=Microaerophilic box not sealed correctly

Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)	Shed No.	Batch No.	Bird No.	Weight of bird, kg	Film Type	Gas compostion at Day 0			Gas compostion at K+3			CFU/g of skin at K+3	log(CFU/g)
					% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2								% O_2	% CO_2	% N_2	% O_2	% CO_2	% N_2		
9	3	41	1.620	BDF	73.6	20.4	6.0	53.4	17.2	29.4	BNS		9	3	101	1.636	SES	18.2	2.7	79.1	14.6	5.0	80.4	2.5	0.40
9	3	42	1.438	BDF	71.0	20.7	9.3	72.0	19.6	8.4	BNS		9	3	102	1.574	SES	20.7	1.7	77.6	14.0	5.1	80.9	17.5	1.24
9	3	43	1.688	BDF	72.7	20.4	6.9	68.4	17.9	13.7	BNS		9	3	103	1.684	SES	19.3	1.7	79.0	14.7	5.2	80.1	27.5	1.44
9	3	44	1.482	BDF	73.9	20.5	5.6	71.6	17.9	10.5	BNS		9	3	104	1.399	SES	20.9	1.5	77.6	16.3	4.1	79.6	12.5	1.10
9	3	45	1.548	BDF	66.8	23.1	10.1	29.8	15.0	55.2	42.5	1.63	9	3	105	1.476	SES	20.9	1.7	77.4	15.6	5.1	79.3	25.0	1.40
9	3	46	1.638	BDF	71.2	19.4	9.4	66.3	19.5	14.2	22.5	1.35	9	3	106	1.418	SES	20.9	1.8	77.3	14.4	5.2	80.4	10.0	1.00
9	3	47	1.522	BDF	73.0	20.1	6.9	20.9	10.3	68.8	127.5	2.11	9	3	107	1.676	SES	20.9	1.9	77.2	13.9	5.4	80.7	72.5	1.86
9	3	48	1.588	BDF	73.4	19.6	7.0	68.8	18.7	12.5	60.0	1.78	9	3	108	1.844	SES	20.9	3.3	75.8	16.0	5.1	78.9	<2.5	0.25
9	3	49	1.566	BDF	74.8	20.4	4.8	62.5	17.9	19.6	20.0	1.30	9	3	109	1.690	SES	20.9	1.8	77.3	13.6	5.4	81.0	<2.5	0.25
9	3	50	1.652	BDF	74.7	20.4	4.9	68.6	19.8	11.6	17.5	1.24	9	3	110	1.500	SES	20.9	1.7	77.4	14.5	4.8	80.7	7.5	0.88
9	3	51	1.866	BDF	75.0	20.0	5.0	68.7	20.5	10.8	12.5	1.10	9	3	111	1.518	SES	20.9	1.7	77.4	14.6	4.9	80.5	80.0	1.90
9	3	52	1.652	BDF	71.1	19.8	9.1	22.4	12.4	65.2	12.5	1.10	9	3	112	1.838	SES	20.9	1.8	77.3	14.0	5.1	80.9	2.5	0.40
9	3	53	1.656	BDF	74.2	20.1	5.7	69.9	18.7	11.4	40.0	1.60	9	3	113	1.678	SES	19.1	1.9	79.0	14.3	5.2	80.5	22.5	1.35
9	3	54	1.860	BDF	74.5	19.6	5.9	42.5	16.2	41.3	10.0	1.00	9	3	114	1.738	SES	20.9	0.5	78.6	14.0	5.3	80.7	162.5	2.21
9	3	55	1.542	BDF	64.3	20.1	15.6	19.6	8.9	71.5	57.5	1.76	9	3	115	1.530	SES	20.9	1.7	77.4	14.1	5.1	80.8	45.0	1.65
9	3	56	1.486	BDF	70.4	19.8	9.8	66.3	18.6	15.1	2.5	0.40	9	3	116	1.462	SES	20.9	1.8	77.3	14.6	5.1	80.3	197.5	2.30
9	3	57	1.706	BDF	67.7	18.4	13.9	22.6	9.8	67.6	27.5	1.44	9	3	117	1.618	SES	20.9	1.8	77.3	14.5	5.1	80.4	280.0	2.45
9	3	58	1.660	BDF	74.8	19.9	5.3	70.0	20.2	9.8	10.0	1.00	9	3	118	1.508	SES	20.9	1.8	77.3	16.0	4.6	79.4	7.5	0.88
9	3	59	1.692	BDF	61.0	19.4	19.6	66.3	19.1	14.6	15.0	1.18	9	3	119	1.634	SES	15.4	1.9	82.7	14.3	5.0	80.7	42.5	1.63
9	3	60	1.466	BDF	72.2	19.7	8.1	23.2	13.2	63.6	37.5	1.57	9	3	120	1.582	SES	19.4	2.0	78.6	15.2	5.1	79.7	190.0	2.28
Mean			1.616		71.5	20.1	8.4	52.7	16.6	30.7		1.35				1.600		20.2	1.8	77.9	14.7	5.0	80.3		1.34
S.D.			0.117		3.8	0.9	3.9	21.1	3.7	24.6		0.40				0.127		1.4	0.5	1.4	0.8	0.3	0.6		0.70
C.I.			0.055		1.8	0.4	1.8	9.9	1.7	11.5		0.19				0.059		0.7	0.2	0.6	0.4	0.1	0.3		0.33
Max			1.866		75.0	23.1	19.6	72.0	20.5	71.5		2.11				1.844		20.9	3.3	82.7	16.3	5.4	81.0		2.45
Min			1.438		61.0	18.4	4.8	19.6	8.9	8.4		0.40				1.399		15.4	0.5	75.8	13.6	4.1	78.9		0.25
Spread			0.428		14.0	4.7	14.8	52.4	11.6	63.1		1.71				0.445		5.5	2.8	6.9	2.7	1.3	2.1		2.20
N			20		20	20	20	20	20	20		16				20		20	20	20	20	20	20		20
n<2.5												0													2

Figure 9. Average oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bars show 95% confidence intervals

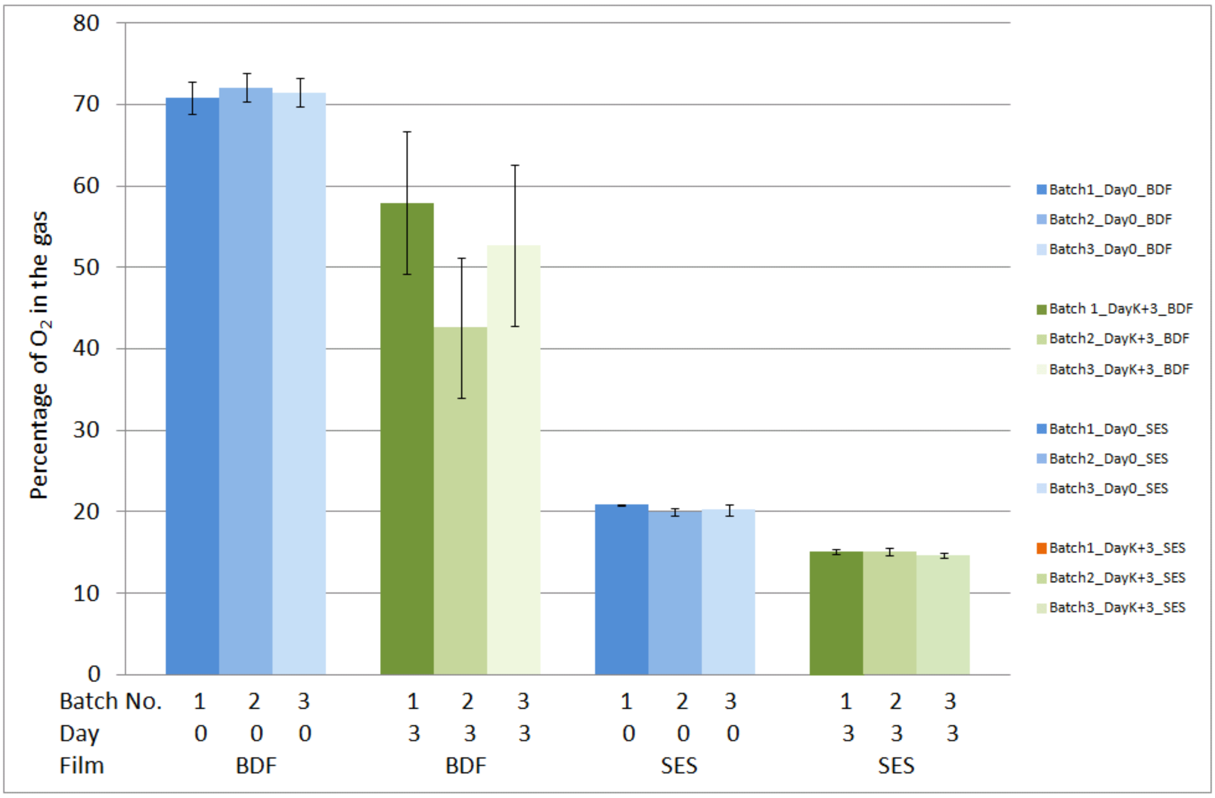
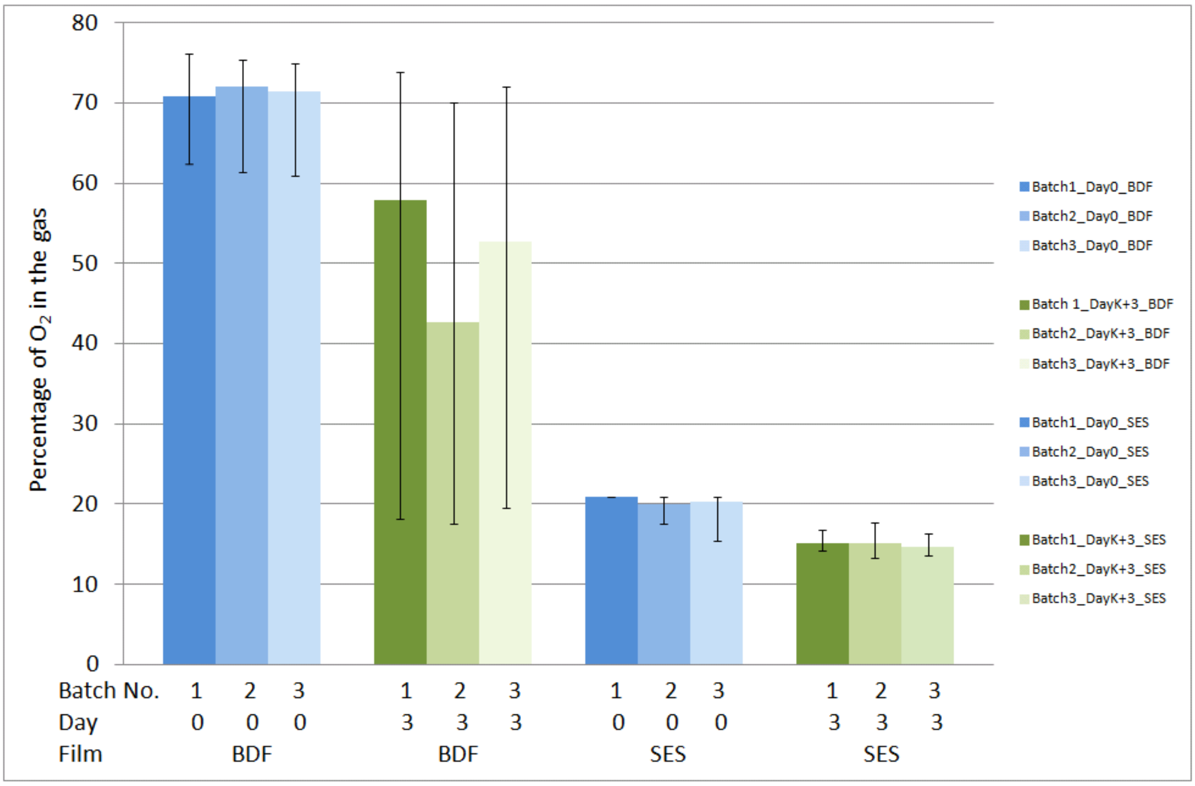


Figure 10. Average, minimum and maximum oxygen concentrations in packs of three batches of birds with O_2 and BDF film or air and SES film on Days 0 and K+3. Error bars show the minimum and maximum values



10. ANALYSIS OF RESULTS FROM OBJECTIVE 4 AND DISCUSSION

Figure 11 shows the numbers of *Campylobacter* on breast skin samples versus the oxygen concentration in the pack head space in the two trials when *Campylobacter* numbers were sufficient to make a judgement on the effect of the gas mix. The results come from the samples packed in air or MA. There is little evidence (p=0.04) at Day K+3 of an effect of oxygen concentration on the numbers of *Campylobacter*.

Figure 11. Numbers of *Campylobacter* (log cfu/g) on breast skin samples versus oxygen concentration in the head space when measured at Day K+3 (Data for birds packed in air or MA)

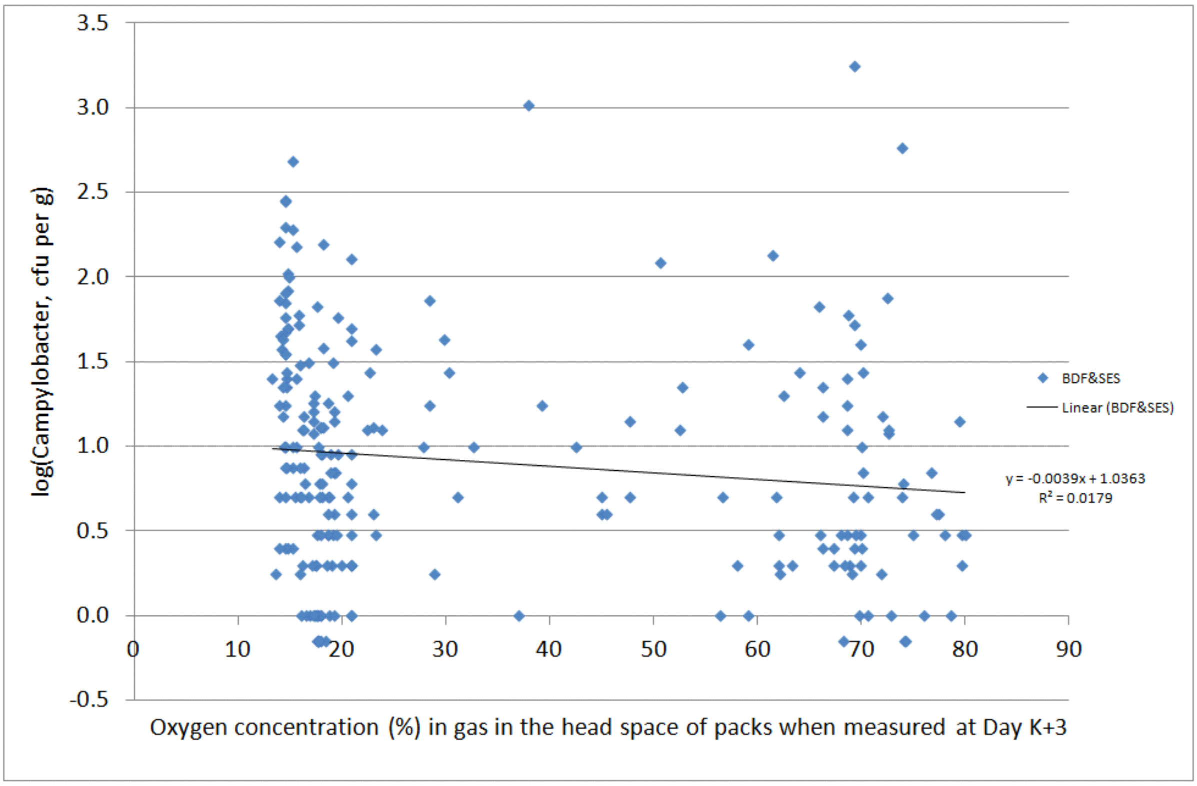
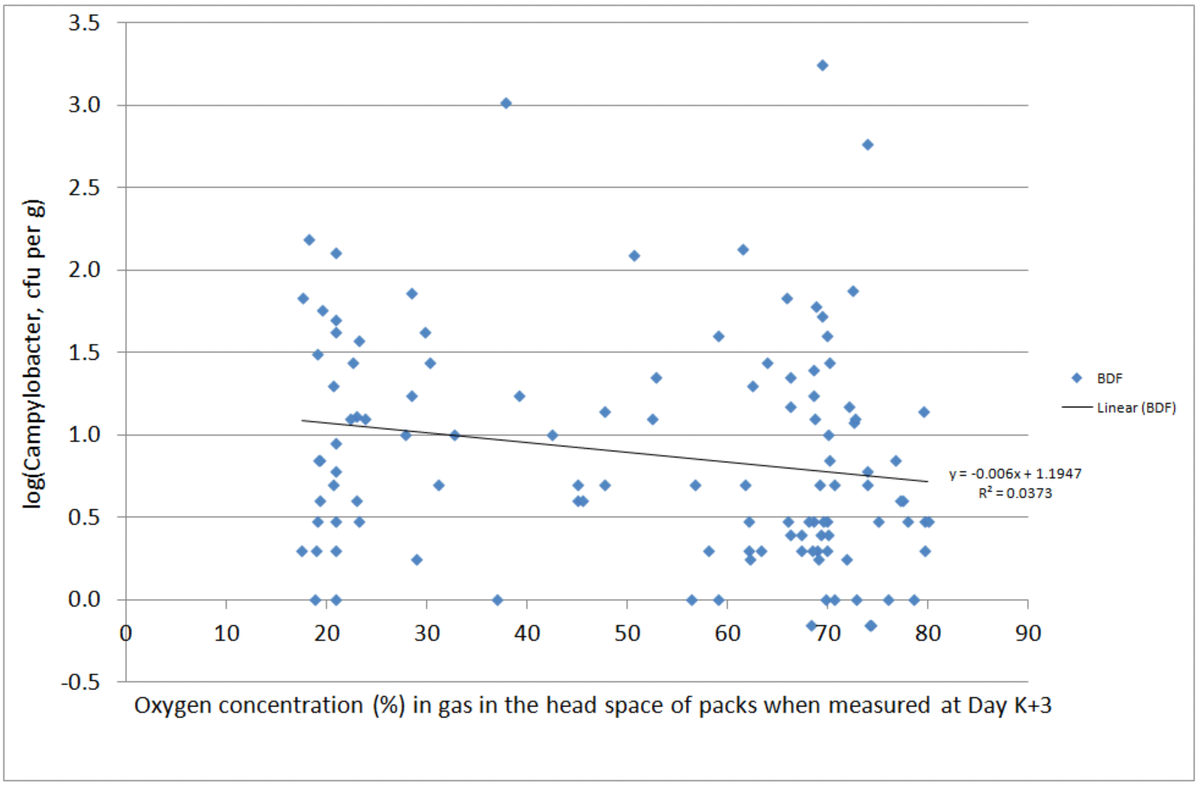


Figure 12 shows the same analysis but using just the data from the MA packs. There is no evidence of a relationship between the gas composition at Day K+3 and the numbers of *Campylobacter*.

Figure 12. Numbers of *Campylobacter* (log cfu/g) on breast skin samples versus oxygen concentration in the head space when measured at Day K+3 (Data for birds packed in MA



There are a number of differences between this study and those reported in the literature which found an effect of gas composition on *Campylobacter*. These include:

(a) Naturally contaminated carcasses were used in these trials whereas spiked carcasses were used in almost all of the work reported in the literature.

(b) In this study, a highly permeable film was used to pack birds in air and a low permeability film was used to pack birds in MA. This is industry practice. In the work in the literature, the same low permeability film was used for birds packed in air or a high oxygen concentration.

(c) A gas:meat ratio of 0.5:1 was use in the current trials as this is common industry practice. The published trials do not specify the gas:meat ratio but, assuming they followed the advice from gas suppliers, the ratio was likely to be about 2:1. Processors report that at this ratio, the packs can appear to be “ballooned” and this is off-putting to consumers who feel that the poultry is suffering bacterial decay. Also, at that size, the packs are large and less items can be put into crates or displayed at retail outlets.

(d) The birds were packed on-line at plants of one processor. Considerable variation in gas composition in the packs was found at Day K+3. Previous published trials were carried out under controlled laboratory conditions.

(e) The birds were tested for *Campylobacter* at Day K+3. This day was chosen because the *Campylobacter* die during storage and beyond Day K+3, the numbers of *Campylobacter* on control or treated birds might have been too low to detect. The effect of the MA might have been greater after a longer storage time. Some processors test at Day K+5 or K+7.

(f) The numbers of *Campylobacter* on the samples in these trials were quite low with most being below 2 $\log_{10}$ cfu/g and many below much lower. With a limit of detection of 5 cfu/g, and assuming a highest count of 100, the highest detectable log reduction due to the treatment would only be 1.5 $\log_{10}$ (i.e. $\log(100) - \log(5/\sqrt{2})$). The numbers of *Campylobacter* were low despite using on-farm testing to identify positive flocks and testing in both spring and summer months.

A possible approach to examining the effect of MA on numbers of *Campylobacter* would have been to measure the gas compositions in the packs being tested as part of the retail survey. However, this was not possible as the *Campylobacter* data was treated as official statistics and so not available for this purpose. Also, there might be differences, other than gas composition, in the ways that the birds had been treated.

The project was discussed at an FSA/Industry Joint Working Group on *Campylobacter - Transport and Processing Subgroup meeting* on 19 January 2015 at which it was agreed that a proposal for taking this project forward should be submitted for consideration. A plan was submitted to the FSA on 4 February which suggested testing birds on Days 0, K+1, K+3, K+7 and K+10 for *Campylobacter* and gas mixture. The trials would be carried out at the sites of a different processor to that used in the previous work. The proposal included trials with portions in addition to whole birds.

On 7 May 2015, the FSA terminated the project with the view that continued funding "would not represent value for money/add to the value of the Agency's *Campylobacter* portfolio". The project, which used samples from just one supplier, had not identified naturally contaminated samples with sufficiently high numbers of *Campylobacter*. To increase the likelihood of obtaining skin samples with higher numbers of *Campylobacter*, the proposed work would have carried out PCR testing of boot swabs from the farms of another processor that had a history of producing birds with higher numbers of *Campylobacter*.

11. CONCLUSIONS

The review of the literature indicated that a high oxygen concentration in-pack, compared to packing in air, would increase the decline of *Campylobacter* numbers during storage. The trials and data gathered in this project indicate that the current industry practice is to pack whole birds in air or in a high oxygen gas mixture (70 or 80% O_2) with a gas:meat ratio of 0.5:1. When using these conditions, no statistically significant evidence was found of an effect on the *Campylobacter* numbers on the birds packed in MA or air. Reasons for the lack of seeing an effect have been suggested including the large variability in gas compositions in the packs at Day K+3, the use of different films for packing in air and MA, and the low initial numbers of *Campylobacter* on the birds.

The project team suggests that the FSA considers measuring gas mixtures in packs used in future retail chicken surveys. This would provide useful information on the variability of the gas mixtures in retail packs and on possibly the effects of MA on numbers of *Campylobacter*.

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Appendix 1 Library of Literature on MA Effects on Poultry

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