MICROBIAL HAZ NTIFICATION OF CHICKEN EGGS PRODUCED BY COMMERCIAL FARMERS IN THE BLOEMFONTEIN REGION

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Central University of Technology, Free State

DECLARATION OF INDEPENDENT WORK

I, HANITA THERON, do hereby declare that this research project submitted to the Technikon Free State for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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OPSOMMING

MIKROBIESE RISIKO-IDENTIFISERING VAN HOENDEREIERS GEPRODUSEER DEUR KOMMERSIëLE BOERE IN DIE BLOEMFONTEIN-OMGEWING

Die uitbreek van siektes wat met gekontamineerde eiers geassosieer word, is 'n wêreldwye verskynsel. Met die hoë voorkoms van MIV/VIGS in Suid-Afrika word dit toenemend belangrik om vas te stel of eiers 'n gesondheidsrisiko vir die verbruiker inhou. Hoewel daar in Suid-Afrika daagliks in die orde van 21 miljoen eiers geproduseer word, is wetgewing met betrekking tot die veiligheid van nie-gepasteuriseerde eiers beperk. In hierdie studie is die mikrobiese gehalte van ewekantsige eiermonsters uit twee verskillende eierproduksie-aanlegte in sentraal Suid-Afrika met mekaar vergelyk. Kontaminasievlakke met *Pseudomonas* spp, *Escherichia coli*, *Salmonella* spp, *Staphylococcus* spp, giste en skimmels is bepaal. Fekaal-geassosieerde bakterietellings in eiers uit die sisteem met 'n gekontroleerde interne omgewing was aansienlik hoër as dié van eiers uit die sisteem waar geen omgewingskontrole toegepas is nie. Mikrobiese tellings van bioaerosolmonsters het hierdie bevinding ondersteun. Na aanleiding van hierdie bevindings is daar by die eierindustrie aanbeveel om eiers te verwyder voordat die fekale materiaal uit die eenhede geskraap word. Betekenisvolle mikrobiese groeipatrone is waargeneem toe eiers tydens rakleeftydstudies aan verskillende temperatuursimulasies onderwerp is. Resultate het getoon dat 'n koue skok van 4 tot 6 uur tydens die vervoer en bewaring van eiers laer bakterietellings tot gevolg gehad het. Om eiergehalte en - veiligheid te bevorder, is daar dus by die eierindustrie aanbeveel om hierdie prosedure in werking te stel.

SUMMARY

MICROBIAL HAZARD IDENTIFICATION OF CHICKEN EGGS PRODUCED BY COMMERCIAL FARMERS IN THE BLOEMFONTEIN REGION

The outbreak of disease associated with contaminated eggs is a worldwide phenomenon. In view of the high prevalence of HIV/AIDS in South Africa, it has become increasingly important to determine whether eggs posed a health risk to the consumer. Though the daily production of eggs in South Africa is in the order of 21 million, legislation regarding the safety of non-pasteurized eggs is limited. In the present study the microbiological quality of randomly selected egg samples from two different egg-producing plants in central South Africa were compared. In the investigation *Pseudomonas* spp, *Escherichia coli*, *Salmonella* spp, *Staphylococcus* spp, yeasts and moulds were tested for. Results showed that fecal-associated bacterial counts were notably higher in eggs from the system with a controlled internal environment compared to eggs from the system where no environmental control was employed. This finding was supported by the microbiological results of the bio-aerosol samples. Based on these findings the recommendation was made to industry that eggs should be removed prior to the scraping of fecal material from the units. Meaningful microbial growth patterns were observed when eggs were subjected to different temperature simulations during shelf-life studies. Results indicated that a cold shock of 4 to 6 hours during transport and storage resulted in lower bacterial counts. To promote egg quality and safety, the implementation of this procedure was therefore recommended to the egg industry.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

1.1 Background to the South African chicken egg industry

In South Africa, the consumption of eggs in recent years has grown considerably. In the period from 1975 to 1985 the growth rate of egg consumption was 20% compared to a growth rate of 57% in the period ranging from 1985 to 1995 (National Department of Agriculture of South Africa). This increasing popularity can most probably be ascribed to the fact that an egg is one of the most economic and abundant food products, has a high satiety value and is easily digested (Kurtzweil, 1998; Papadopoulou *et al.*, 1997; Mountney, 1989). In 2001 the average South African per capita consumption of chicken eggs was estimated at 5.8 kg (approximately 116 eggs), while the number of commercial egg producers amounted to 550 (National Department of Agriculture of South Africa). According to Van der Westhuizen (Head of the School: Environmental Development and Agriculture, Technikon Free State [2002]) a commercial farmer has to produce more of a certain product than he requires and must sell part of his produce to make a profit. Almost all egg producers in South Africa deliver directly to the retail industry or sell directly to the consumer. On estimate 96% of eggs consumed are sold in shell form, while the remaining 4% are sold as egg products to bakeries and the meat industry amongst others.

According to Wethli (1999) egg production in Southern Africa can be categorised as backyard chickens, home egg production, (small) business or layer management (larger business). The National Department of Agriculture states that the egg industry

in South Africa comprises of three distinct sectors, namely day-old chick production, layer replacement hen production and egg production.

In South Africa commercial farmers use two typical high-rise systems in egg production: the uncontrolled high-rise layer system and the controlled high-rise layer system.

The uncontrolled high-rise layer system

An uncontrolled high-rise layer system (Fig. 1.1) is a double-level structure characterised by a construction boasting five rows of cages equipped with an automated egg removal mechanism. In a high-rise housing system the cage configuration for laying hens is arranged in such a way that manure is dropped through an open space between and beneath the cages to the lower level. The fecal matter disposal mechanism feature has a central opening in the floor through which the matter drops directly from each bird to an open-air lower level. The fecal matter is removed on an infrequent basis, but usually once a year.

The A-frame manure curtain cage configuration (Donald, 1997) permits the highest possible density for a high-rise type house and minimises space between cages. The manure curtain is placed behind each cage to prevent manure droppings from contacting birds in cages below. The system is also equipped with extractor fans at the one end of the top level and air vents in the roof; no other environmental control systems are present. This system is enclosed with a sheet-metal roof and walls. All cages are equipped with automated feeders, waterers and fill systems.

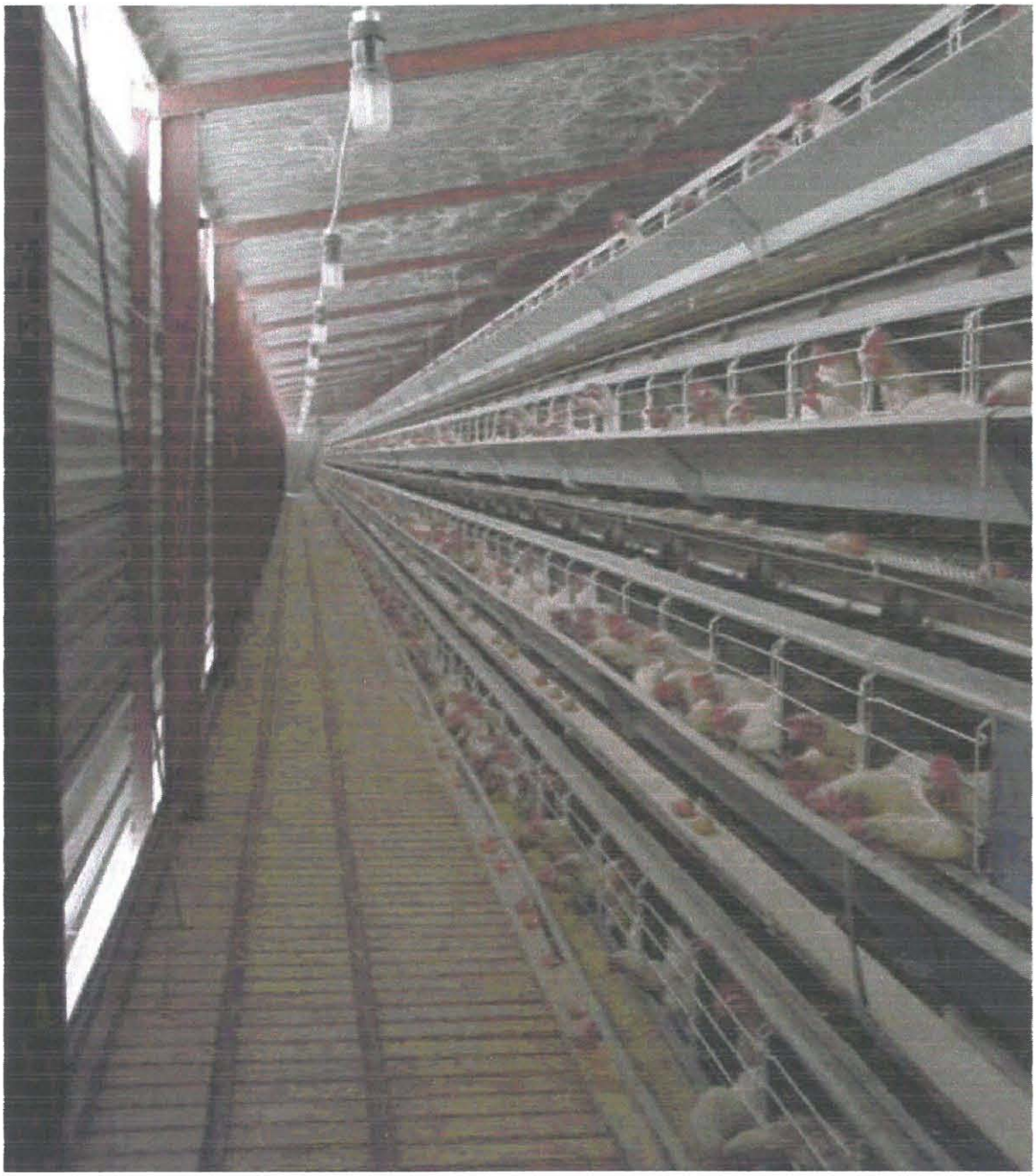


Fig. 1.1 Highline Silvers in an uncontrolled high-rise system at a commercial chicken egg farm.

The controlled high-rise layer system

The controlled high-rise layer system (Fig. 1.2) features similar characteristics as the uncontrolled system except that it is a single level structure enclosed with smooth brick walls. Similarly, it contains five rows of cages equipped with an automated egg removal mechanism. The controlled system is also equipped with an automated fecal removal mechanism.

This fecal removal mechanism contains conveyer belts set up below each cage set. Every morning the conveyer belt, which forms part of the mechanical scraper, scrapes and removes the fecal matter from the belt at the one end of the chicken cages. This system is further equipped with an automated environmental control system that regulates the temperature and humidity with water-cooled filters embedded in the walls at the one end and extraction fans on the other.

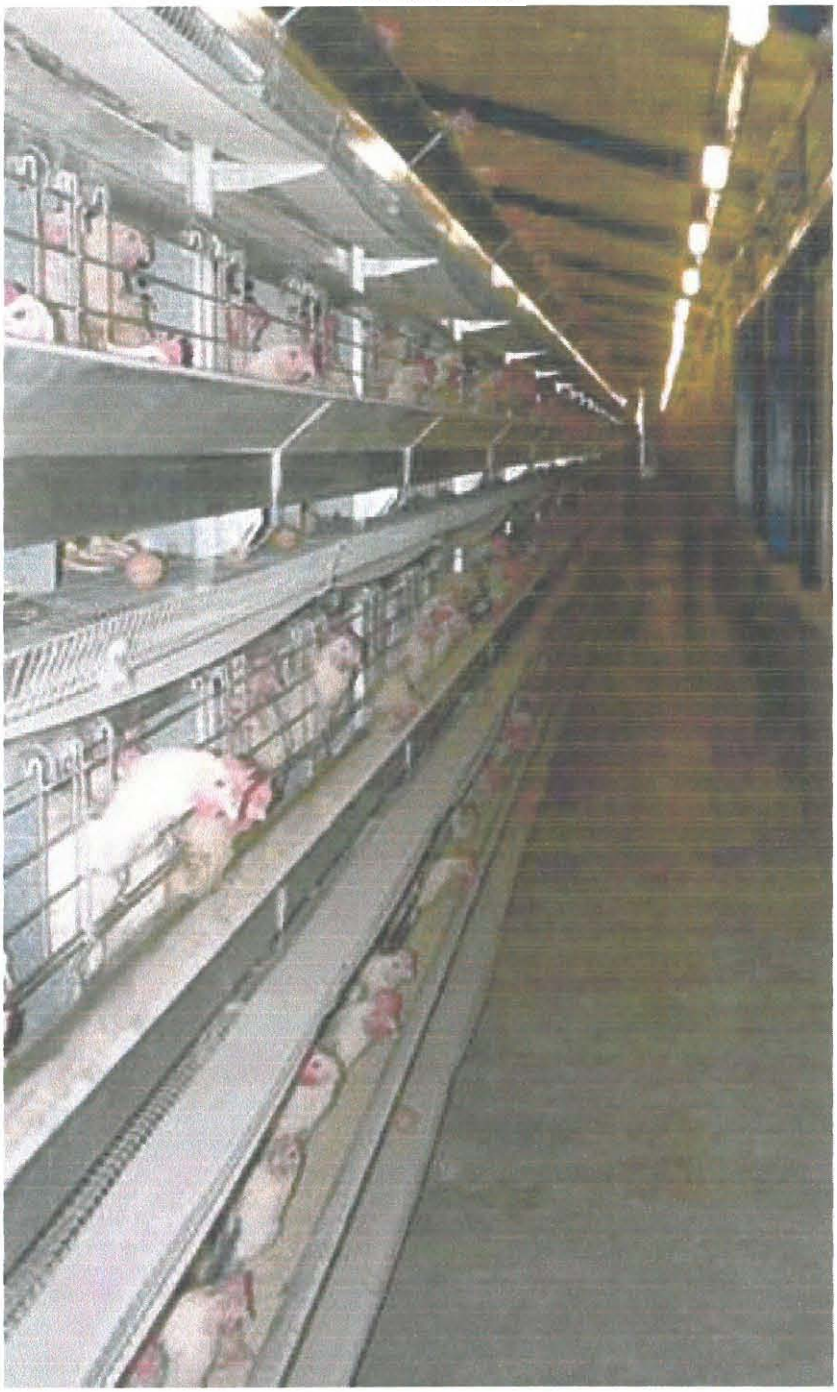


Fig. 1.2 Highline Silvers in a controlled high-rise system at a commercial chicken egg farm.

1.2 Hazard identification of chicken eggs

Hazard identification forms part of the risk assessment process and HACCP. This process is a scientific approach to estimate the risks and factors involved in food production. Hazard identification consists of the identification of biological, chemical and physical agents capable of causing adverse health effects that may be present in a particular food or group of foods (Forsythe 2000 b). In 2002 the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations followed this process to assess the risks posed by *Salmonella enteritidis* contaminated eggs. Foods of animal origin, especially poultry, poultry products and raw eggs, were implicated in sporadic cases and outbreaks of human salmonellosis (WHO; 2002).

According to Grijspeerdt (2001), Murase *et al.* (2001) Forsythe and Haynes (1998) there have been an enormous increase in salmonellosis in many parts of the world. The evolution of the *Salmonella enterica* serotype Enteritidis pandemic beginning in the 1980s led to increased foodborne illnesses associated with poultry in many countries, specifically outbreaks and single cases associated with eggs and egg products (WHO, 2002; Thorns, 2000). Chang (2000) estimates that internal contamination with *Salmonella* spp may occur in 0.01% to 0.6% of all poultry eggs in the USA. Ching-Lee *et al.* (1991) have found the external contamination of commercially purchased poultry eggs in Hawaii to be as high as 9%. Jay (2000) refers to the largest salmonellosis outbreak in 1994 in the United States, which involved more than 224,000 persons. The vehicle food was ice cream produced from milk that was transported in tanker trucks that had previously hauled liquid eggs. Thorns (2000)

summarized international data that provides estimated incidences of salmonellosis per 100 000 people for the year 1997: 14 in the USA, 38 in Australia, and 73 cases per 100 000 in Japan. In the European Union, the estimates range from 16 cases per 100 000 (The Netherlands) to 120 cases per 100 000 in parts of Germany.

1.3 The biochemistry and microbiology of chicken eggs

Eggs are highly perishable products, mainly because of their high nutritional value (Papadopoulou *et al.*, 1997; Bennion, 1990). With their high water content, eggs are ideal for microbial growth as microorganisms are dependent on the presence of water (Schlegel, 1995; Potter, 1986). Although the egg has various ways to protect itself against microbial invasion, undesirable storage temperatures and excessive contamination may permit bacteria to penetrate the eggshell and cause spoilage (Frazier and Westhoff, 1988).

1.3.1 Biochemical composition and functionality

The chemical composition of a large, whole egg without its shell is listed in Table 1.1 (Bennion, 1990). The entire egg consists of the following components: water (73.6%) and solids (26.4%) divided into organic and inorganic matter (International Commission on Microbiological Specifications for Foods [ICMSF], 1980). The shell consists mainly of calcium carbonate (94%), magnesium carbonate, calcium phosphate and organic matter (Mountney, 1989). The protein in the egg white contains all the essential amino acids, making egg protein one of the highest quality proteins. Eggs are therefore considered as one the most important human nutrition sources; in fact, nutritionists use the egg as a standard of reference against other food proteins (Wang and Slavik, 1998;Mountney, 1989).

Table 1.1. The average chemical composition of one large whole egg (50g) without its shell.

Chemical components	Quantity
Water	75 %
Energy	80 kilo calories
Protein	6 gram
Fat	6 gram
Iron	1.0 milligram
Vitamin A	260 International Units
Thiamin	0.04 milligram
Riboflavin	0.15 milligram

(Bennion, 1990)

Bennion (1990) states that egg proteins are of excellent nutritional quality and have the highest protein efficiency ratio of all common foods, meaning that eggs very nearly match the amino acid requirement of the human body. Mountney (1989) furthermore states that eggs have all the essential vitamins (except vitamin C), which include the water-soluble vitamins (the vitamin B complex), and the fat-soluble vitamins (A, D, E and K).

The various methods applied in general egg preparation (for human consumption) are poached, soft-boiled (in shell), hard-boiled (in shell), fried, scrambled, stirred, omelettes, crepes, soufflés, custards and meringues (Bennion, 1990). Not only are eggs prepared as mentioned, but they also act as a major ingredient in many processed foodstuffs for instance baked goods, mayonnaise and soups thanks to their many functional properties (Mountney, 1989).

According to Mountney (1989) these functional properties include leavening (in baked goods eggs are mainly responsible for the textures of breads, cakes and other bakery products), binding (to hold other ingredients together, e.g. croquettes), thickening (e.g. cream soup), retardation of sugar crystallisation (prevents gritty texture in cakes icing and candies), emulsification (egg yolk contains natural emulsifiers like lecithin), clarification (aspic), coating or glazing (preventing dehydration, but giving a firm glistening coat e.g. for baked products) and colouring or garnishing food.

1.3.2 Microbiology of chicken eggs

1.3.2.1 Microbial ecology

According to Banfart (1989) microorganisms can inhibit or stimulate one another's growth and because all living forms have as their main goal self-preservation and the continuation of the species, microorganisms have to compete for food and other necessities. As a result, various actions and reactions (or co-actions) that may be detrimental, neutral or beneficial to one or all species involved may influence the microbial ecology in an environment.

All microorganisms follow similar growth patterns and are affected by the microbiota of the environment and a combination of the critical growth factors. Inside the egg the microorganisms with less nutritional selectivity will grow faster than organisms which are fastidious in their requirements (Zeidler, 1995). The growth of a bacterial culture can be visualised graphically by plotting the logarithms of cell numbers or viable counts against time (Schlegel, 1995). In a favourable environment a typical sigmoid-shaped growth curve forms, which can be divided into growth phases as

shown in Fig. 1.3. These are the lag phase, exponential or logarithmic phase, stationary and death phase. The growth phases are usually present but may appear more or less strongly expressed (Schlegel, 1995) due to the influence of various growth and inhibitory factors.

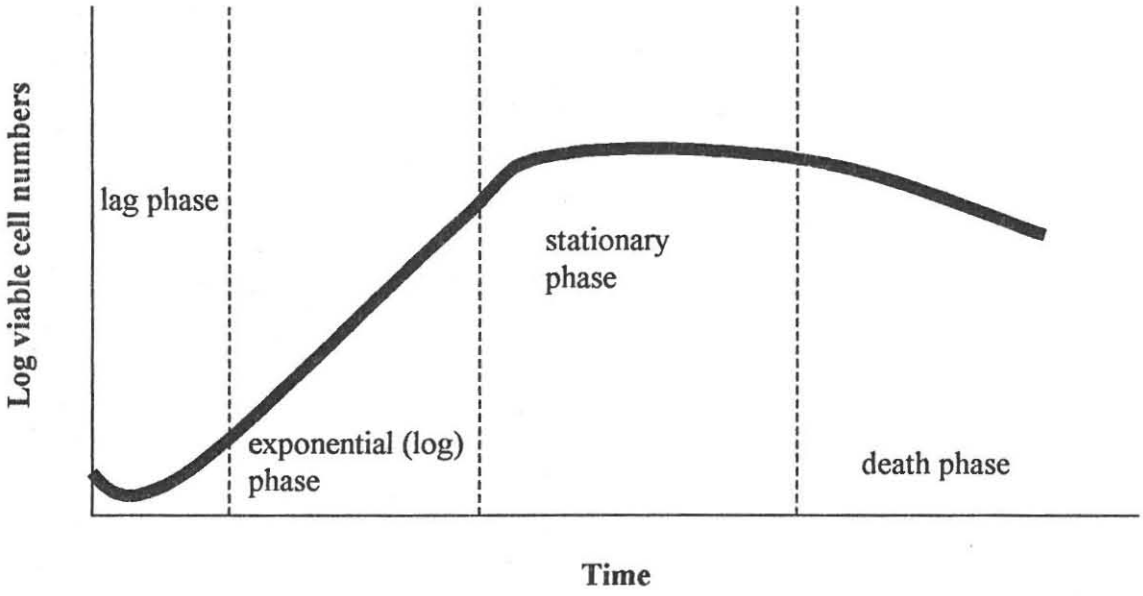


Fig. 1.3 A hypothetical growth curve of a bacterial culture in a favourable environment.

According to Zeidler (1995) growth factors that influence microbial growth in eggs are divided into intrinsic and extrinsic factors.

- Intrinsic: All the factors associated with the egg itself, such as the nutrients, water, water activity, pH and presence of growth inhibitors as well as competing microorganisms are included in this group. Schlegel (1995) reports that the growth of microorganisms is dependent on the presence of water, which makes eggs ideal for microbial growth. Egg proteins is highly nutritious as previously

mentioned (Bennion, 1990). Mountney (1989) also states that eggs include ample quantities of the previously mentioned vitamins (Table 1.1). All of these will support microorganism growth, and in particular important members of the families Enterobacteriaceae (*Escherichia, Klebsiella* and *Shigella*) and Pseudomonadaceae which are responsible for food spoilage at high water activity (a_w) (Shapton and Shapton, 1991).

- Extrinsic: This group includes all the factors associated with the surrounding environment of the egg, such as temperature, relative humidity and presence or lack of oxygen. Most Salmonellae (the most prominent pathogen), as well as *Escherichia coli* and *Staphylococcus aureus* proliferate well above 15.6°C in liquid egg leading to the emphasis on the refrigeration of eggs (ICMSF, 1980). Jay (2000) further states that the spoilage of eggs by microorganisms is favoured by high humidity.

1.3.2.2 Contamination of eggs

Major routes of food contamination include: cross-contamination of raw and processed food products; inadequate control of temperature during storage, cooking and cooling; poor personnel hygiene and inadequate monitoring of manufacturing processes (Forsythe, 2000 a). In addition to this Lutgring *et al.* (1997) add bioaerosols (viable airborne contaminants from a biological source) as a prominent source of food contamination in various industries. In fact, it has become increasingly important to make use of air sampling for the characterisation of the microbiota

within the food production environment to evaluate product quality and safety (Lutgring *et al.*, 1997).

The consumer should assume that any poultry product has a degree of bacterial contamination on or in it (Bjerklie and Riley, 1990). This is disturbing, because it is important that eggs be free from microbial contamination as they are used in the preparation of many foodstuffs for human consumption. An egg's principal function is to be a reproducing entity, providing the necessary nourishment for the developing chick until hatching (21 days for the domestic chicken), and providing protection against microbial penetration from a generally soiled and polluted environment (ICMSF, 1980). With egg formation the yolk passes down the oviduct of the hen and the white is added before the whole becomes covered with a shell (Nickerson and Sinskey, 1972). According to Nickerson and Sinskey (1972) it is possible that eggs contain no viable bacteria when laid, but considering the manner in which they are formed, there is nevertheless some chance of bacterial contamination of the egg. Furthermore, eggshells are moist when laid and are therefore easily soiled which increases the chance of contamination (Pienaar, 1995). Jay (2000), however, comments that the majority of freshly laid eggs are sterile.

Egg-producing facilities are usually confined structures, which are densely stocked with egg-laying hens that create large volumes of dust and manure. According to Zeidler (1995) these facilities are microbiologically extremely active owing to processing activities and poor personal hygiene of employees. The viable airborne microorganisms that originate from egg residues, feathers and dirt further accumulate and are aerosolized easily. It is therefore safe to conclude that the eggshell harbours a

large number of bacteria as a result of contamination with, amongst other things, faeces, dust and feeding material (Harrigan and McCance, 1976).

Nickerson and Sinskey (1972) contend that less than 1% of naturally clean eggs become spoiled bacteriologically during egg formation, which indicates that most bacteria get into the egg from the outside environment. Bacterial contaminants most frequently found on eggs, according to Jay (2000), include members of the genera *Pseudomonas, Bacillus, Escherichia, Enterobacter, Proteus, Staphylococcus* and *Salmonella*, the latter is the only major human pathogen carried by eggs according to the ICMSF (1980). Moulds associated with eggs are members of the genera *Mucor, Penicillium, Hormodendron* and *Cladosporium* amongst others, while *Torula* is the only yeast found with any degree of consistency (Jay, 2000).

In Table 1.2 the morphology and the optimum, maximum and minimum temperatures to sustain growth of *Staphylococcus aureus, Pseudomonas* spp, serotypes of *Escherichia coli* and *Salmonella* spp are listed. The associated habitats of these microorganisms include:

- *Staphylococcus aureus*: generally moist food will support the growth of *Staphylococcus aureus*. Humans are the most important source of *Staphylococcus aureus*. *Staphylococcus* can grow to large numbers without causing changes in the odour, taste or physical appearance of food (Nickerson and Sinskey, 1972).

- *Pseudomonas* spp: these non-pigmented species is the psychotropic bacteria most often associated with the spoilage of refrigerated fresh foods (Jay, 2000).

- *Escherichia coli*: six serotypes can be transmitted in different foods, especially those associated with poultry or poultry products. Their main habitat is human and animal intestines (Forsythe and Haynes, 1998).

- *Salmonella*: Salmonellae are widely distributed in nature, with humans and animals as their main reservoirs (Jay, 2000).

Table 1.2 Morphology and temperature parameters for *Salmonella* spp, serotypes of *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus aureus.*

Bacteria	Morphology	Infective dose, parameters for development and destruction temperature
Salmonella spp	Gram-negative rods usually motile with peritrichous flagella. Short (0.5-0.7µm in width and 1-3µm in length). Aerobe and facultative anaerobe.	1.5-9.1·100g^{-1} cheese Temperature; pH Minimum: 5.1°C; 4.0 Optimum: 37 °C; 6.5-7.5 Maximum: 45°-47°C; 9.0 Destroyed at 60°C in 15-20 min.
Serotypes of *Escherichia coli*	Gram-negative facultative anaerobic rods, short (1.1-1.5 µm x 2.0-6.0 µm) and motile. Catabolise carbohydrates and reduce nitrates.	Infective dose ranges from 1 to not exceeding 100g^{-1} or ml^{-1}of food product. The optimum growth temperature: 37°C Minimum under 15°C Destroyed at 70°C for 2 minutes, but toxin is resistant to 100°C for 15 min.

(Table continues on p.26)

Bacteria	Morphology	Infective dose, parameters for development and destruction temperature
Pseudomonas spp	Gram-negative, motile rods 0,3-0.5 x 1.0-4.0 µm in size. Usually aerobes, catalase and oxidase positive, and deaminate arginine. Some species attack carbohydrates oxidatively.	Less than $1 \cdot 100 g^{-1}$ of product The optimum growth temperature: between 20°C to 25°C, although many *Pseudomonas* are capable of growth well below 0°C. Destroyed at 55°C.
Staphylococcus aureus	Gram-positive cocci, spherical or ovoid in shape. 0.5-1µm in diameter. Non-motile, characteristic grape-like clusters are seen when examined microscopically. Aerobe and facultative anaerobe	10^6 cells per g cause food poisoning. The minimum quantity of enterotoxin needed to cause illness in humans is about 20 ng Temperature; pH Minimum: 11°C; 4.0 Optimum: 37°C; 6-7 Maximum:45°-47°C; 9.8-10 Destroyed at 66°C for 10 min., but the toxin survives 100°C for 30 min.

(Jay, 2000; Forsythe and Hayes, 1998; Shapton and Shapton, 1991)

1.3.2.3 Shelf-life and safety of chicken eggs

According to South African legislation governing egg production (Act 54 of 1972), an egg product after pasteurisation or irradiation shall conform to the following microbiological specifications:

- *Salmonella* organisms shall be absent in $25 \cdot gram^{-1}$ or $25 \cdot ml^{-1}$ of an egg product;
- *Staphylococcus aureus* shall be absent in $1 \cdot gram^{-1}$ or $1 \cdot ml^{-1}$ of an egg product;
- Mesophilic aerobic bacteria shall not exceed 20 000 colony-forming units $(cfu) \cdot gram^{-1}$ or 20 000 $cfu \cdot ml^{-1}$;

- Coliforms shall not exceed $50 \cdot gram^{-1}$ or $50 \cdot ml^{-1}$ of an egg product;
- Yeasts and moulds shall not exceed $200 \cdot gram^{-1}$ or $200 \cdot ml^{-1}$ of an egg product.

This legislation was promulgated to control the microbiological quality of eggs. With the country suffering from unemployment, poverty and illiteracy, however, the focus has moved away from law enforcement and toward education and sustainability.

The definition of shelf-life according to Man (2002) is the period of time under defined conditions of storage, after manufacturing or packaging, for which a food product will remain safe and be fit for use. During this period the food product should retain its desired sensory, chemical, physical, functional or microbiological characteristics. Because all foods deteriorate and may then host pathogenic bacteria, every food product has a microbiological shelf-life. Safety and shelf-life of food are therefore related (Man, 2002).

With regard to food safety Schultze and Fawcett (1996) state that *Salmonella* spp have been found to contaminate poultry eggshells. The shell's contents can be contaminated from the external environment through cracks in the shell or by transovarian passage of organisms from the chicken to the egg. According to Wang and Slavik (1998) "horizontal" bacterial contamination (when organisms on the eggshell penetrate into the egg interior and contaminate the egg contents) of eggs appears to be the major route. These results present a serious cause for concern as the Centers for Disease Control and Prevention (CDC) (1996) report that *Salmonella enteritidis'* food poisoning is a virulent and sometimes fatal infection.

According to the CDC (1996) egg consumers should exercise caution when using raw eggs in cooking. One of their guidelines stipulates that those who are ill or have compromised immune systems, such as people with Human Immunodeficiency Virus (HIV) and the elderly, should avoid raw eggs at all times (CDC, 1996). Other guidelines by the CDC (1996) in using raw eggs in cooking include the buying of refrigerated eggs and keeping them refrigerated at less than 5 °C; the cooking of eggs for at least 15 seconds at 60°C (the white should be completely set and the yolk beginning to thicken); the eating of eggs soon after cooking and the washing of hands, cooking surfaces and utensils after contact with raw eggs. Hospitals, nursing homes and commercial kitchens should use pasteurised eggs rather than unpasteurised eggs.

1.3.2.4 Natural defence mechanisms of the egg

According to Jay (2000), Frazier and Westhoff (1988) and the ICMSF (1980) there are at least two factors responsible for limiting microbial penetration and proliferation amongst eggs.

- The resistance to microbial penetration of the shell and its membranes: according to Jay (2000), Wang and Slavik (1998) and Pienaar (1995) a fresh egg has three structures externally and each is in some degree effective in restricting the entry of microorganisms: the outer, waxy shell membrane (cuticle), the shell and the inner shell membrane (Fig. 1.4). The cuticle acts by closing the pores within the shell covering to prevent bacterial penetration (Wang and Slavik, 1998). The shell serves as a physical barrier to prevent bacterial penetration of the egg (Jay,

2000; Wang and Slavik, 1998; Pienaar, 1995). The inner and outer membranes, which are made up of many interwoven protein filters acting as a bacterial filter, separate only at the blunt end of the egg (Wang and Slavik, 1998; Pienaar, 1995). The shell structures are, in decreasing order of ability to restrict penetration of microbes, the cuticle → the inner membrane → the shell → the outer membrane (Fig. 1.4). Todd (1996) states that cracks that penetrate the inner membrane allow microorganisms to bypass the barriers and permit immediate entry of spoilage and pathogenic bacteria. In this case, if a shell is heavily soiled, the chance that bacteria will penetrate sooner and in greater numbers is elevated (ICMSF, 1980). If the cuticle of the egg is damaged, there is an even greater frailty to microbial entry. According to Frazier and Westhoff (1988) bacteria can further enter the shell when a drop in temperature occurs. The reduction in temperature causes the air sac to contract and to draw water and bacteria through the shell and against the inner membrane. As the air sac grows through ageing this effect becomes more evident. This may be one of the factors that make stored eggs more susceptible to penetration. Antibacterial activity of the membranes was also reported and can be traced to the presence of lysozyme in both membranes (ICMSF, 1980).

- The multiple factors in egg white that provide undesirable conditions for bacterial proliferation: according to ICMSF (1980) there are multiple factors in the albumin that prevent microbial penetration and proliferation in eggs. The albumin (Fig. 1.4) inhibits or prevents growth of a wide variety of microorganisms, while the egg yolk remains inert or non-active in the battle against microorganisms. Table 1.3 summarises the main factors in egg white that yield undesirable conditions for bacterial proliferation.

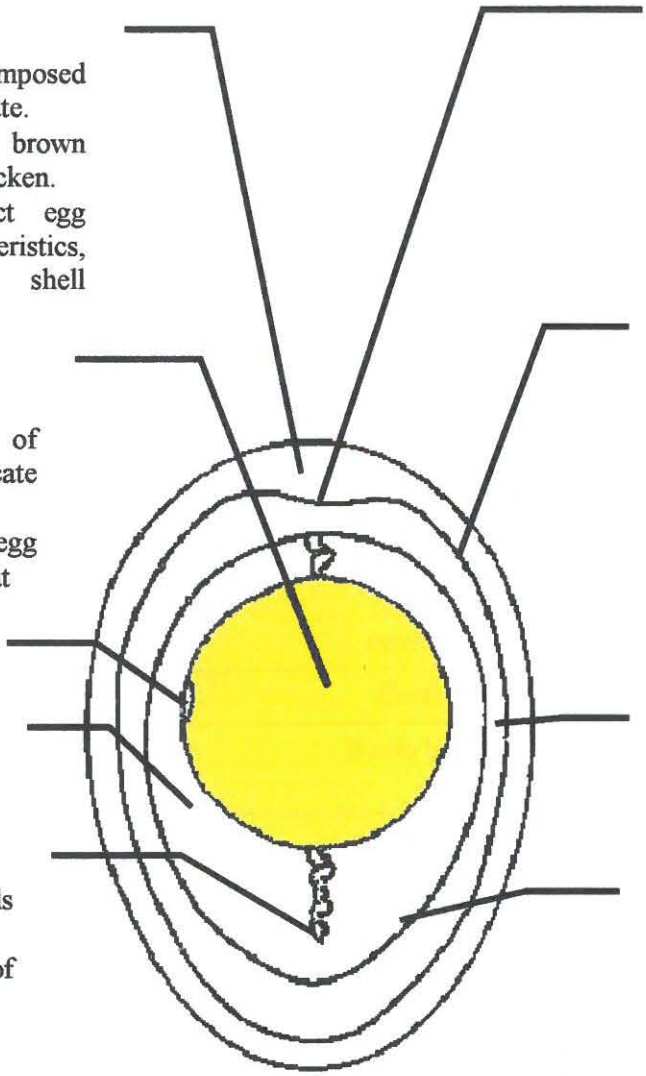
Shell
- Outer covering of egg composed largely of calcium carbonate.
- May be white or brown depending on breed of chicken.
- Colour does not affect egg quality, cooking characteristics, nutritional value or shell thickness.

Yolk
- Yellow portion of egg
- Colour varies with feed of the hen, but doesn't indicate nutritional content.
- Major source of egg vitamins, minerals, and fat

Germinal Disc

Vitelline (Yolk) Membrane
- Holds yolk contents

Chalazae
- Twisted, cordlike strands of egg white
- Anchor yolk in centre of egg.
- Prominent chalazae indicates freshness



Air Cell
- Pocket of air formed the large end of egg.
- Caused by contraction the contents duri cooling after laying.
- Increases in size as e ages

Shell Membranes
- The inner and outer shell membranes surround the albumen (two membranes)
- Provide protective barrier against bacterial penetration
- Air cell forms betweer these two membranes.

Thin Albumin (White)
- Nearest to the shell
- Spreads around thic white of high-quality egg

Thick Albumin
- Major source of egg riboflavin and protein
- Stands higher and spreads less in higher-grade eggs.
- Thins and becomes indistinguishable from thin white in lower grade eggs.

Fig. 1.4 A schematic representation with the composition and primary functions of individual components of a chicken egg (American Egg Board, 2001).

Table 1.3 The multiple antimicrobial factors in albumin that provide undesirable conditions for the proliferation of microorganisms in eggs.

Component	Activity
Lysozyme (muramidase: because it attacks murein layer of bacteria cells)	Lysis of cell walls of Gram-positive bacteria. Flocculation of bacteria cells. Hydrolysis of β-1,4-glycosidic bonds.
Conalbumin	Chelation of iron, copper and zinc, especially at high pH.
Riboflavin	Chelation of cations.
Glucose	Oppression of respiratory capacity of facultative anaerobes – some uncertainties still occur.
pH 9.1-9.6*	Improve chelating potential of conalbumin. Provides unsuitable environment for many organisms.
Low non-protein nitrogen	Certain organisms cannot grow.
Avidin	Binds biotin, making it unavailable to bacteria that require it.
Apoprotein	Combines with riboflavin.
Ovoinhibitor	Inhibits fungal proteases.
Ovomucoid	Inhibits trypsin, but does not affect growth of Gram-negative bacteria.
Uncharacterised proteins	Inhibit trypsin and chymotrypsin. Combines with vitamin B6, chelate calcium and inhibits ficin and papain.

*The pH of a newly laid egg white is 7.6 – 7.8. The pH rises to 9.1 – 9.6 after a few days at room temperature, during which CO_2 evolves (Jay, 2000; Frazier and Westhoff, 1988; ICMSF, 1980).

1.4 Rationale

With egg-associated food-borne disease outbreaks occurring globally it becomes important in a country with high numbers of individuals with AIDS or HIV to determine whether eggs pose a threat to consumers. South Africa has limited legislation on unpasteurised eggs and has a daily production of about 21 million eggs. The production sites for these eggs are usually confined and extremely congested buildings, which in general are likely to contribute to end product contamination. This leads to the following research questions: are there microbial hazards present in unpasteurised eggs produced by commercial farmers in the central Free State region (Chang, 2000; Schultze and Fawcette, 1996; Ching-Lee *et al.*, 1991) and when eggs are subjected to temperature abusive conditions during transport and storage, does the initial microbial contamination on the egg influence egg quality and shelf-life?

This study focuses on microbial hazard identification that will be performed at a commercial egg-producing farm in the central Free State region. Microbial egg quality, safety and shelf-life will be investigated by simulating conditions of varying temperature. The reason for this is that temperature abuse might occur during storage and transport of chicken eggs and might have an effect on the bacterial contamination and proliferation in and on eggs. With this as background the objectives of this study were:

1) to assess and compare the microbial quality of eggs from two different egg layer systems on a commercial egg farm by conducting hazard identification for the presence of *Salmonella* spp, *Staphylococcus* spp, *Pseudomonas* spp, *Escherichia coli*, yeasts and moulds on and in chicken eggs (Chapter 2).

2) to investigate the possible source/s of the microorganisms found on the eggs This will be done through quantification and distribution of the viable airborne microorganisms (bioaerosols) in both a controlled environment and an uncontrolled environment egg layer system (Chapter 3).

3) to determine whether bacterial growth patterns, shelf-life and safety of eggs, with specific reference to the total aerobic plate counts (APC), *Salmonella* spp, *Escherichia coli*, *Staphylococcus* spp, *Pseudomonas* spp and Total coliforms will be affected by various storage and transport conditions (Chapter 4).

Data generated in this study will be applied to make recommendations to the egg industry in order to improve egg quality and egg shelf-life in the interest of consumers.

1.5. References

American Egg Board. 2001. *The Incredible Edible Egg* website: Frequently asked questions about eggs: Composition Chart [Online] as viewed on February 19, 2002. http://www.aeb.org/professional/productinfo/shelleggs.html#grading.

Banfart, G.J. 1989. Factors that affect Microbial Growth *In*: Banfart, G.J. ed. *Food in basic food microbiology*. (2nd ed.) (pp. 101-163) Van Nostrand Reinhold: New York.

Bennion, M. 1990. Eggs and egg cookery *In*: Bennion, M. ed. *Introductory foods*. (9th ed.) (pp. 273-300) Macmillan Publishing Company: New York.

Bjerklie, D. and Riley, M. 1990. The dangers of foul fowl *Time* 136, (23) 78

Centers for Disease Control and Prevention. 1996. Outbreaks of *Salmonella* serotype *enteritidis* infection associated with consumption of raw shell eggs - United States, 1994-1995. *Morbidity and Mortality Weekly Report* 45, (34) 737-742.

Chang, Y.H. 2000. Prevalence of *Salmonella* spp in poultry broilers and shell eggs in Korea. *Journal of Food Protection* 63, (1) 655-658.

Ching-Lee, M.R., Katz, A.R., Sasaki, D.M. and Minette, H.P. 1991. *Salmonella* egg survey in Hawaii: evidence for routine bacterial surveillance. *American Journal of Public Health* 81, (6) 764-766.

Donald, J. 1997. Planning for commercial layer expansion or renovation. *Poultry Housing Information Series* 701(8), 6-9.

Forsythe, S.J. 2000 a. Foodborne Illness In: Forsythe, S.J. ed. The microbiology of safe food. (pp. 53-95) Blackwell Science: Oxford.

Forsythe, S.J. 2000 b. Microbial Risk Assessment In: Forsythe, S.J. ed. The microbiology of safe food. (pp. 335-360) Blackwell Science: Oxford.

Forsythe, S.J. and Hayes, P.R. 1998. *Food hygiene, microbiology and HACCP.* (3rd ed.) Aspen Publishers, Inc.: Maryland.

Frazier, W.C and Westhoff, D.C. 1988. *Food Microbiology.* (4th ed.) McGraw-Hill Book Company: New York.

Grijspeerdt, K. 2001. Modelling the penetration and growth of bacteria in eggs. *Food Control.* 12, 7-11.

Harrigan, W.F. and McCance, M.E. 1976. Microbiological examination of specific foods *In:* Harrigan, W.F. and McCane, M.F. eds. *Laboratory Methods in Food and Dairy microbiology.* (pp. 168-9) Academic Press: London.

International Commission on Microbiological Specifications for Food (ICMSF) 1980. Eggs and Egg Products *In*: Silliker, J.H., Elliot, R.P., Baird-Parker, A.C., Bryan,

F.L., Christian, J.H.B., Clark, D.S., Olson, J.C., Jr. and Roberts, T.A. eds. *Microbial Ecology of Foods*. Vol II (pp. 521-566). Academic Press: New York.

Jay, J.M., 2000. Miscellaneous food products. *In*: Jay, J.M. ed. *Modern Food Microbiology*. (6th ed.) Aspen publishers, Inc.: Maryland.

Kurtzweil, P. 1998. Safer eggs: laying the groundwork. *FDA Consumer* 32, (5) 10-16.

Lutgring, K.R., Linton, R.H, Zimmerman, N.J. Peugh, M and Heber, A.J. 1997. Distribution and quantification of bioaerosols in poultry-slaughtering plants. *Journal of Food Protection* 60, (7) 804-810.

Man, D. 2002. *Shelf life*. Blackwell Science: Oxford.

Mountney, G.J. 1989. *Poultry Products Technology*. (2nd ed.) Food Products Press: New York.

Murase,T., Senjya, K., Maeda,T., Tanaka, M., Sakae, H., Matsumoto, Y., Kaneda, Y., Ito, T. and Otsuki, K. 2001. Monitoring of chicken houses and an attached egg-processing facility in a laying farm for *Salmonella* contamination between 1994 and 1998. *Journal of Food Protection* 64, (12) 1912-1916.

National Department of Agriculture of South Africa. 2001. The Resource Centre; Directorate: Agricultural Information Services P/Bag X144 Pretoria. Website:

National Department of Agriculture of South Africa: Statistical Information: Trends in the Agricultural Sector 2001: Poultry: Egg Industry [Online] as viewed on November 3, 2002. http://www.nda.agric.za

Nickerson, J.T. and Sinskey, A.J. 1972. *Microbiology of foods and food processing*. American Elsivier Publishing Company: New York.

Papadopoulou, C., Dimitriou, D., Levidiotou, S., Gessouli, H., Panagiou, A., Golegou, S. and Anatoniades,G., 1997. Bacterial strains isolated from eggs and their resistance to currently used antibiotics: is there a health hazard for consumers? *Comparative Immunology, Microbiology and Infectious Diseases* 20, (1) 35-40.

Pienaar, A.C.E., Coetzee, L. and Bragg, R.R. 1995. A rapid method to determine bacterial contamination on hatching eggs. 2. Correlation of the optical-density measurements after incubation to bacterial counts on hatching eggs. *Onderstepoort Journal of Veterinary Research* 62, 25-33.

Potter, N.N., 1986. Deteriorative factors and their control. *In*: Potter, N.N. ed. *Food Science*. (4th ed.) (pp.140-168) Van Nostrand Reinold: New York.

Schlegel, H.G. 1995. Growth of microorganisms *In*: Schlegel, H.G. *General microbiology*. (7th ed.) (pp.193-233). Cambridge University Press: Great Britain.

Schultze, G.E. and Fawcette, H.A. 1996. Prevalence of *Salmonella enteritidis* in poultry shell eggs in Arkansas[*]. *Southern Medical Journal* 89, (9) 889-892.

Shapton, D.A. and Shapton, N.F. 1991. *Principles and practices for safe processing of foods*. Butterworth and Heinemann Ltd.: Oxford.

South Africa. 1972. *Foodstuffs, Cosmetics and Disinfectants Act, 1972 No. R.692 of 16 May 1997.* Government Printers: Pretoria.

Todd, E.C.D. 1996. Risk assessment of use of cracked eggs in Canada. *International Journal of Food Microbiology* 30, 125-143.

Thorns, C.J. 2000. Bacterial food-borne zoonoses. *Revenue scientifique et technique Office international des epizooties*, 19(1): 226–239.

Van Der Westhuizen, C. 2002. Personal communication. 12 February 2002. Bloemfontein.

Wang, H and Slavik, M.F. 1998. Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. *Journal of Food Protection* 61, (3) 276-279.

Wethli, E. 1999. *The Southern African chicken book*. Juta and Co.,Ltd.: Kenwyn.

World Health Organization/Food and Agriculture Organization of the United Nations. 2002. Risk assessments of Salmonella in eggs and broiler chickens. Microbial

risk assessment Series 2. *Food and Agriculture Organization of the United Nations*. [Online] as viewed on May, 30 2003. ftp://ftp.fao.org/es/esn/food/RA_Salmonella_report.pdf

Zeidler, G. 1995. What makes microorganisms grow on eggs and in the egg room? (Part I). *Poultry Bulletin* 12, 363-365.

Chapter 2

MICROBIAL HAZARD IDENTIFICATION OF CHICKEN EGGS PRODUCED IN HIGH-RISE LAYER SYSTEMS

Submitted for publication to: ***Food Control***

2.1 Abstract

The present study was conducted to compare and to assess the microbial quality of eggs from two different egg layer systems by conducting microbial hazard identification. Twenty eggs from each of a controlled and an uncontrolled high-rise layer management system (LMS) were collected. Each egg was washed in 50 ml sterile Nutrient Broth, and broths from five egg washings were combined and analysed for the microbial content of the eggshells according to various selective and differential media. The contents of the five eggs were subsequently combined in 500 ml Nutrient Broth after the shells had been sterilised by submersion in alcohol. The specified media were finally used to identify the microbiota of the egg contents. In the controlled system, higher *Escherichia coli* and *Salmonella* spp counts were found on the eggshells, whereas in the uncontrolled system higher mold and yeast counts, as well as higher total aerobic plate counts (APC) and *Pseudomonas* spp counts were enumerated. With the exception of the yeasts and moulds in the egg contents from the controlled LMS, the contents of the eggs from the uncontrolled system were found to be the more contaminated of the two. The eggshells were however, more contaminated in the uncontrolled system, with the exception of *E. coli* and coliforms. The higher fecal-related contamination on the shells in the controlled LMS is attributed to the automated fecal removal mechanism.

2.2 Introduction

Chicken eggs are one of the most economical protein foods available. They are easily prepared and appetising, and are characterised by a high satiety value and are also easily digestible (Kurtzweil, 1998; Mountney, 1989). Eggs are consumed by the majority of the population in South Africa, including pregnant women, elderly people, bottle-fed infants, those with weakened immune systems, and persons with liver disease, who are more susceptible to food-borne disease (Key, 1996). According to the Resource Centre Directorate Agricultural Information Services of the National Department of Agriculture of South Africa (2001), the average *per capita* consumption of chicken eggs consumed in South Africa during 2001 was estimated at 5.8 kg (approximately 116 eggs). Little or no information is presently available on the microbial hazards associated with chicken eggs in South Africa.

Food-borne disease is caused by the consumption of eggs contaminated with biological agents and/or toxins present in quantities equal to or exceeding the minimum infectious dose (Frazier and Westhoff, 1988). An example of such a disease-causing pathogen associated with the consumption of eggs and egg dishes is *Salmonella enteritidis*, which is the causative agent of salmonellosis. The incidence of this pathogen has increased in many parts of the world in recent times and has become an international health concern (Grijspeerdt, 2001; Evans *et al.*, 1998; Humphrey and Whitehead, 1992). Particular attention has been paid to the role of chicken eggs in the transmission of *Salmonella* to the human populations in Europe (Lee, 2000), Canada (Todd, 1996) America (Trepka *et al.*, 1999; Schultze and Fawcette, 1996; Ching-Lee *et al.*, 1991), Kuwait (Kamel *et al.*, 1980), Korea (Chang, 2000) and Poland (Radkowski, 2001).

An egg-producing facility is microbiologically extremely active, as processing activities often result in drainage overflow, excessive water condensation and dripping, and large amounts of egg residues, feathers and dirt are generated. Unhygienic practices such as spilled water, unclean employee clothing and boots, uncontrolled head or facial hair, and especially unwashed hands and poor personal hygiene further contribute to microbial hazards (Zeidler, 1995). It is also claimed that the density of the chicken population within a high-rise building creates large volumes of dust and manure, liberally contaminating all surfaces in the house (Kreager, 1999).

In addition to the above-mentioned sources of contamination, chicken eggs, when heavily contaminated with "old" microbiota (organisms that are well adapted to the intrinsic antimicrobial factors of eggs) constitute a source where such organisms may proliferate. Ziedler (1995) reported that microbial proliferation occurs easily on proteinaceous foods such as eggs, meat and blood. Bacterial contaminants most frequently found on eggs, according to Jay (2000), include members of the genera *Pseudomonas, Bacillus, Escherichia, Enterobacter, Proteus, Staphylococcus* and *Salmonella* (the latter is the only major human pathogen carried by eggs according to the International Commission On Microbiological Specifications For Foods) (ICMSF, 1980). Contamination by microorganisms may also occur during the development of the egg in the oviduct (the canal through which the egg travels) of an infected hen or from fecal matter coming into contact with an egg after laying (Kurtzweil, 1998).

Several research projects have been conducted on chicken eggs in various parts of the world to determine the microbial quality of chicken eggs (Grijspeerdt,

2001;Radkowski, 2001; Chang, 2000; Lee, 2000; Trepka *et al.*, 1999; Evans *et al.*, 1998; Schultze and Fawcette, 1996; Todd, 1996; Humphrey and Whitehead, 1992; Ching-Lee *et al.*, 1991; Kamel *et al.*, 1980). In South Africa, many studies relating to hazard identification have been conducted on milk, meat and poultry, while little research has been conducted on eggs, even though eggs pose a substantial threat (Chang, 1999; Trepka *et al.*, 1999; Shultze and Fawcett, 1996; Ching-Lee *et al.*, 1991). In this study the focus had been placed on the two typical high-rise systems utilised by commercial farmers, namely a controlled layer management system (LMS) (a system isolated from the external environment, boasting internal temperature and humidity control) and an uncontrolled LMS (a system not completely isolated from the external environment, with no control over temperature or humidity). The high-rise layer system is a regularly employed facility design, which, according to Donald (1997), is the arrangement of cages of laying hens in such a manner that the manure is dropped through an open space between and beneath the cages. Donald (1997) reports that high-rise housing can be used successfully in different climatic conditions, with the major key to success being the ventilation system. The author furthermore mentions that egg quality, production and uniformity are related to temperature and air quality (Donald, 1997).

The present study therefore addresses the qualification and quantification of potential microbiological hazards associated with chicken eggs produced on a farm in central South Africa. This study outlines the influence of differences in facility design on the microbial population on these eggs, obtained from both a controlled and an uncontrolled internal environment layer management system (LMS). This study was conducted in an attempt to cast light on the microbial hazards observed both on the

shell and within the contents of chicken eggs and to make recommendations on how to lower these microbial hazards.

2.3 Materials and methods

2.3.1 Sampling Site

The uncontrolled LMS

This system consists of a dual-level structure characterised by a construction with five rows of cages equipped with an automated egg removal mechanism. In this high-rise housing system the cage configuration for laying hens is arranged in such a way that manure drops through an open space between and beneath the cages to the lower level (Fig. 2.1). The fecal matter disposal feature has a central opening in the floor through which the matter drops directly from each bird to an open-air lower level. This fecal matter is removed once a year. The A-frame with manure dropping board cage configuration permits the maximum possible density for a high-rise type house whilst minimising space between cages (Fig. 2.1). The manure dropping board is placed behind each cage to prevent manure droppings from coming into contact with birds in the cages below. The system is furthermore equipped with extractor fans at one end of the top level as well as air vents in the roof; no other environmental control systems are present. This system is enclosed by sheet-metal roof and walls, and each cage is equipped with an automated feeder, a waterer and a fill system.

The controlled LMS

The controlled LMS features characteristics similar to the uncontrolled system, except that it is a single-level structure enclosed by smooth brick walls. This system is equipped with an automated fecal removal mechanism boasting conveyer belts set up

below each cage set, removing the fecal matter each morning. The controlled system further employs a fully automated environmental control unit, which regulates the temperature and humidity by means of water-cooled filters embedded in the walls at one end, and extraction fans at the other.

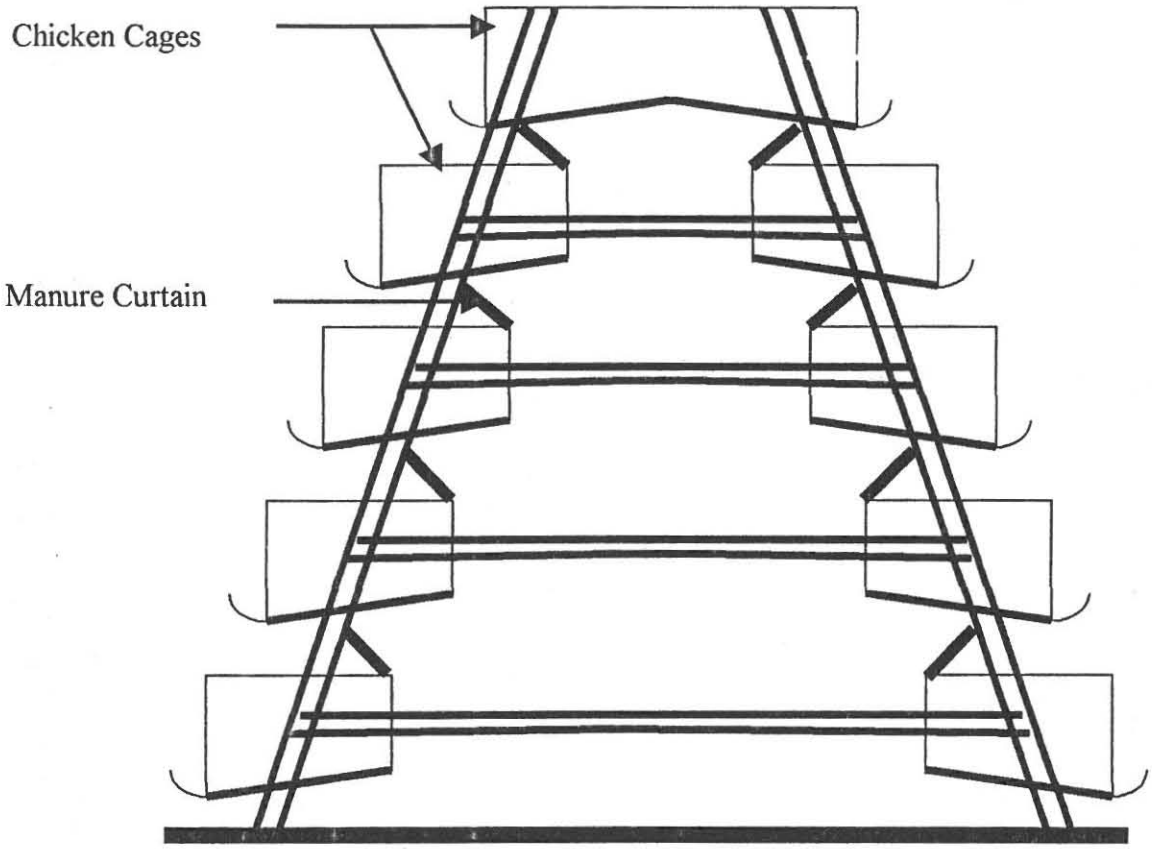


Fig. 2.1 A typical A-frame with manure curtain found in high-rise layer systems (Donald, 1997).

2.3.2 Sampling

The study was conducted on a commercial farm that employs both the controlled and the uncontrolled LMS. Both systems house the same species of chicken (Highline Silvers), of the same age and from the same distributor, which are fed the same food

at the same time. Random samples were collected on Monday mornings when eggs from the layer houses of a specific system were mixed. Twenty eggs were collected from each system (n=20 for each system). The eggs were placed in packaging cartons, labelled and stored below 5°C to be examined in the laboratory later that day.

2.3.3 Egg analysis

The analysis of the eggs consisted of two steps:

i) the analysis of the microbial contaminants on the eggshell (egg intact); and

ii) the analysis of the microbial contaminants associated with the egg contents.

Eggshell

A modification of the egg washing procedure (method of removing contaminants from the eggshell) used by Alaboudi *et al.* (1988), Pienaar *et al.* (1995) and Gentry and Quarles (1972), was used to wash the eggs. Each egg was gently washed in 50 ml sterile Nutrient Broth in a sterile plastic bag by rubbing the surface of the eggs, through the plastic bag, for 30 seconds (Pienaar *et al*, 1995). The broths from five egg washings were consequently combined and analysed (Alaboudi *et al.*, 1988).

Egg contents

After washing, the eggshells were sterilised by submerging them briefly in alcohol, and then drying them in a sterile cabinet. The contents of five eggs were combined by cracking open the eggs aseptically and emptying the liquid content into 500 ml sterile Nutrient Broth, after which the liquid was shaken until evenly mixed (Schultze and Fawcette, 1996).

For both the eggshells and their contents, serial dilutions were prepared with the use of saline solution (Biolab-SA). The streak plate method was further applied to quantify the various microbial groups (Herbert, 1990) using the following agars: Plate Count Agar (PCA, MERCK, Martley *et al.*, 1970) followed by incubation at 25°C for 48 hours for the enumeration of the total aerobic plate counts (APC); Cetrimide Agar with added glycerol (MERCK, Goto and Enomoto, 1970) followed by incubation at 25°C for 18-48 hours for *Pseudomonas*; Baird-Parker Agar (MERCK, Nikanen and Aalto, 1978) followed by incubation at 36°C for 48 hours for *Staphylococcus* spp; Chromocult Coliform Agar (MERCK, Manafi and Kneifel, 1989) followed by incubation at 36°C for 48 hours for Total Coliform and *Escherichia coli*; and Potato Dextrose Agar (PDA, MERCK, Beever and Bollard, 1970) followed by incubation at 25°C for 5 days for yeasts and moulds.

For *Salmonella* spp 10 ml of the Nutrient Broth from the egg mixture was transferred to 100 ml of Rappaport Vassiliadis medium (SCHARLAU, Vassiliadis, 1983, Hammack *et al.*, 2001) immediately after mixing, followed by incubation at 42°C for 24 hours. One loop of sample was consequently transferred to Brilliant Green Agar plates (BGA) (SCHARLAU, Osborn and Stokes, 1955) and incubated for 24-48 hours at 42°C.

2.3.4 Data analysis

All samples were analysed at least in duplicate and $P \leq 0,05$ was used as limit of significance. For normally distributed data Excel for Windows was used for analysis of significance (P).

2.4 Results and discussion

Low counts of *Pseudomonas* spp were observed on the eggshells from the uncontrolled LMS. On the shells of the eggs from the uncontrolled system, growth varied between 0-7.5 cfu per 5 eggs, with no growth detected in the contents. No *Pseudomonas* spp was detected on the shells or in the contents of eggs from the controlled system (Table 2.1). *Staphylococcus* spp was detected on the shells as well as inside eggs from both LMS systems. Eggs from both LMS systems had higher *Staphylococcus* spp counts on the shells compared to the contents. Eggs from both LMS systems showed no significant difference ($P \leq 0,05$) between the microbial counts of the shells and that of the contents (Tables).

A significant difference was, however, observed between the shells and the contents of eggs from the uncontrolled system (P=0.0001) when comparing the moulds and yeasts. Mold counts obtained from the shells of eggs from the uncontrolled LMS system, namely 138.8 cfu per 5 eggs, were relatively high considering that moulds were not detected in the contents of these eggs. This marked difference may be ascribed to the airflow from the open-air lower fecal catchment level towards the eggs, which acts as a vector for the fungal spores (Venter *et al.*, 2003). These results also differ from results obtained from eggs from the controlled LMS where no significant difference was observed between the contamination of the shell and the egg contents.

Table 2.1 Comparison of the microbial content of eggshells and contents in an uncontrolled layer management system.

	Uncontrolled LMS				
	Shell			Contents	
	Mean ($\bar{x}$)	Standard Deviation (SD)	Significance (P)	Mean ($\bar{x}$)	Standard Deviation (SD)
Total aerobic plate counts (APC)	3077.5	±3333.8	0.0001	137.5	±105.7
***Pseudomonas* spp**	2.5	±5.0	0.0030	0.0	±0.0
***Staphylococcus* spp**	183.8	±95.7	0.1500	41.3	±37.1
Moulds and Yeasts	138.8	±193.7	0.0001	0.0	±0.0
Total coliforms	158.8	±223.1	0.0230	41.3	±48.7
Escherichia coli	125.0	±230.1	0.0090	25.0	±33.2
	Presence after 48 hours enrichment			**Presence after 48 hours enrichment**	
Salmonella* spp	0%			100%	

*The percentage *Salmonella* spp on / in the mentioned eggs does not indicate the quantity of this organism on / in the eggs. The sample analysed was a mixture of 5 eggs / sample followed by an enrichment step (section 2.3.3) which preceded the *Salmonella* spp absence / presence evaluation.

Table 2.2 Comparison of the microbial content of eggshells and contents in a controlled layer management system.

	Controlled LMS				
	Shell			Contents	
	Mean ($\bar{x}$)	Standard Deviation (SD)	Significance (P)	Mean ($\bar{x}$)	Standard Deviation (SD)
Total aerobic plate counts (APC)	1012.5	±156.6	0.0630	41.3	±43.3
***Pseudomonas* spp**	0.0	±0.0	1.0000	0.00	±0.0
***Staphylococcus* spp**	86.3	±40.7	0.1160	30.00	±14.1
Moulds and Yeasts	87.5	±175.0	0.3810	50.0	±100.0
Total coliforms	503.8	±1007.5	0.0001	12.5	±9.6
Escherichia coli	460.0	±920.0	0.0001	7.5	±5.0
	Presence after 48 hours enrichment			**Presence after 48 hours enrichment**	
Salmonella* spp	100%			75%	

*The percentage *Salmonella* spp on / in the mentioned eggs does not indicate the quantity of this organism on / in the eggs. The sample analysed was a mixture of 5 eggs / sample followed by an enrichment step (section 2.3.3) which preceded the *Salmonella* spp absence / presence evaluation.

The enumeration of possible fecal contaminants, namely Total coliforms, *E. coli* and *Salmonella* spp revealed differences concerning the level of fecal contamination on the shells compared to that of the egg contents. These counts also differed markedly between the two systems under investigation. Levels of contamination on the shells of eggs from the uncontrolled LMS were in general higher than those from the controlled LMS. The fecal contaminants, on the other hand, were present in higher numbers on eggs from the controlled LMS. This difference could be attributed to the automated fecal matter disposal system in the controlled LMS which, according to Venter *et al.* (2003), scrapes fecal matter from the conveyer belt, producing high levels of aerosolized particles of fecal origin that contaminate the eggshells and the egg handling machinery (e.g. egg elevators and conveyer belts).

Immediate analysis of the eggs from both egg production systems yielded no Salmonella spp counts. This conforms to current South African legislation (act 54 of 1972), which states that no Salmonella spp should be present in 25 ml of pasteurized eggs. For the purpose of this study the eggs were further enriched to reveal the absence or presence of dormant Salmonella spp (Tan & Shelef, 1999; Andrews et al., 1984). In egg samples from the uncontrolled system 100% of the contents tested positive for the presence of this organism, with none detectable on the shells. In contrast, eggshells from the controlled system had 100% Salmonella spp presence with a 75% presence in samples from the contents (Tables).

The contamination of egg contents by Salmonella spp was also investigated by Ching-Lee et al. (1991) who reported that egg contents could be contaminated with *Salmonella* spp due to the penetration of this organism through cracks in the shell.

Internal contamination can also occur prior to shell formation through transovarian transmission (Ching-Lee *et al.*, 1991). Since counts of bacteria that usually are associated with fecal contamination were relatively high on the eggshells, this could indicate a possible source of the fecal-associated organisms that were detected in the egg contents, while chickens from both systems seemed to suffer from a degree of internal contamination. Chicken feed is considered as another possible route for introducing *Salmonella* infections into poultry flocks (Davies *et al.*, 1997). Lee (2000) furthermore mentions that flocks infected with *Salmonella enteritidis* rarely present symptoms.

Comparing the two systems

The total aerobic plate counts (APC) of organisms on the shells of eggs from the uncontrolled LMS was significantly higher than that of the contents, (P=0.0001; Table 2.1). This differs from results obtained in eggs from the controlled LMS where an overall microbial concentration three times lower was observed, with no significant difference between the microbial concentration on the shell and within the contents.

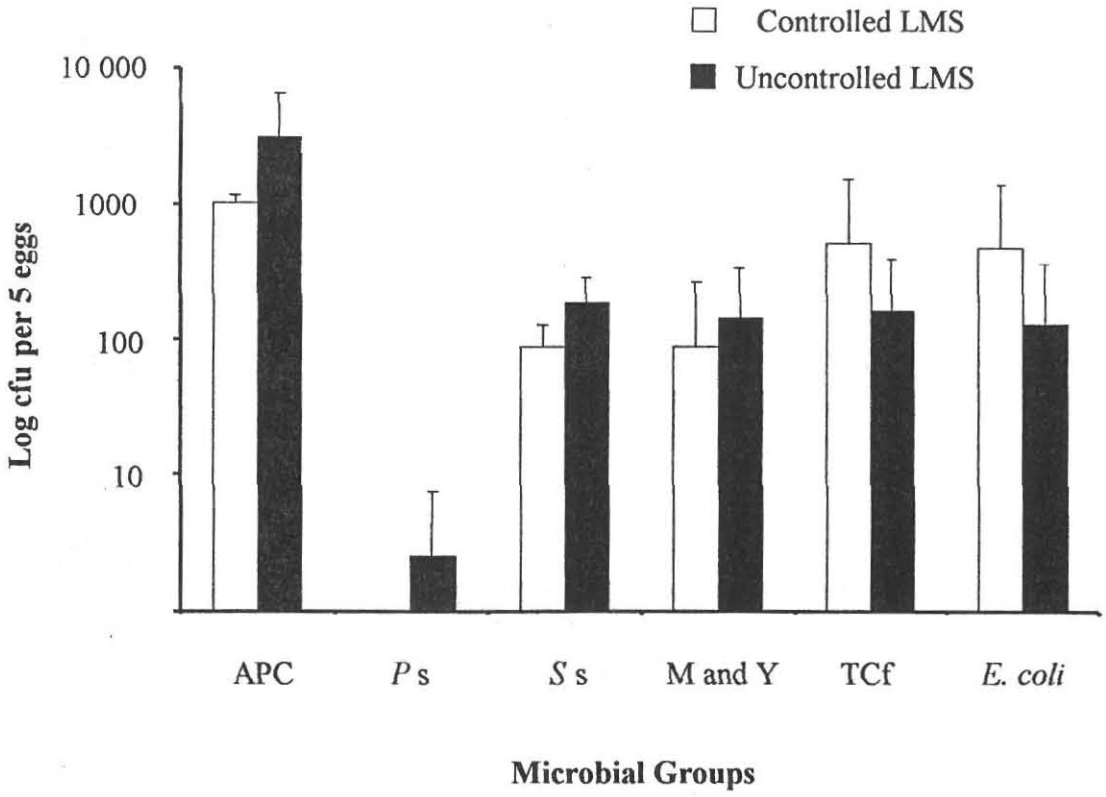


Fig. 2.2 A comparison of the *Pseudomonas* spp (*P*s), *Staphylococcus* spp (*S*s) moulds and yeasts (M and Y), Total Coliform (TCf), *Escherichia coli* (*E. coli*) and total aerobic plate counts (APC) found on the eggshells from the controlled and the uncontrolled layer management system.

When comparing the microbial counts of organisms on the eggshells of eggs from both the layer management systems (Fig. 2.2) it was evident that the growth was higher on the eggshells for all the organisms except the Total coliforms and *E. coli*. No *Pseudomonas* spp was detected on the eggshells from the controlled LMS. A significant difference in microbial counts between the eggshells of the controlled and the uncontrolled systems was observed (total APC, P=0.0035; *Pseudomonas* spp, P=0.0033; Total coliforms, P=0.0345; and *E. coli*, P=0.0476).

When comparing the microbial load of the contents of eggs from both of the layer management systems (Fig. 2.3) the uncontrolled LMS presented higher levels of contamination than the controlled LMS, particularly with regard to the total APC, *Staphylococcus* spp, Total coliforms and *E. coli*. No *Pseudomonas* spp was detected in the contents of eggs from either LMS.

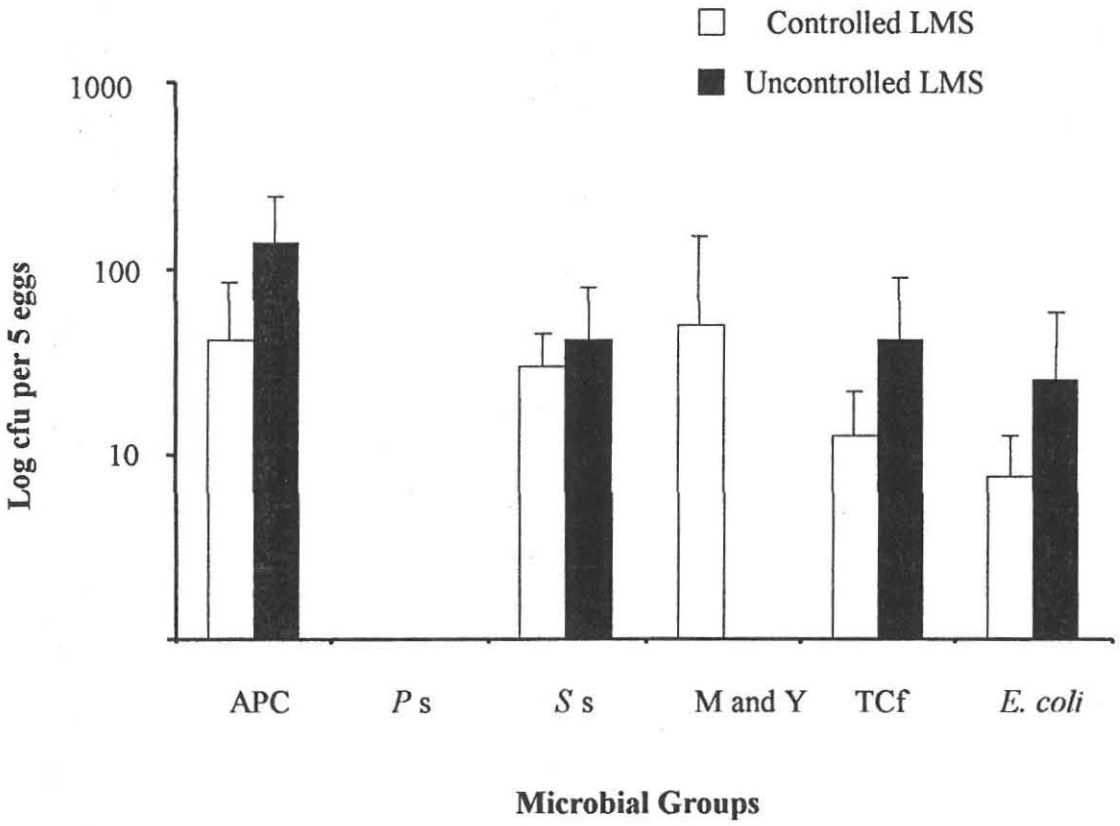


Fig. 2.3 A comparison of the *Pseudomonas* spp (*P*s), *Staphylococcus* spp (*S*s) moulds and yeasts (M and Y), Total Coliform (TCf), *Escherichia coli* (*E. coli*) and total aerobic plate counts (APC) found in the egg contents from the controlled and the uncontrolled layer management system.

In conclusion, findings from the present study suggested that eggs from both eggs from both LMS systems contained relatively low levels of spoilage organisms as opposed to Total coliforms, *E.coli*, yeast and moulds. Based on the *Slamonella* spp, Total coliforms and *E.coli* counts, the contents of eggs from the uncontrolled LMS system appears to be more contaminated. Unrestricted airflow introducing fungal spores into the atmosphere of the uncontrolled LMS system probably accounted for the higher fungal counts detected on the shells of eggs from this system. The relatively high microbial counts on eggshells from the controlled LMS system could have resulted from aerosolisation of fecal material during the scraping of fecal material. Contamination probably resulted when aerosolised fecal particles and microorganisms associated with it had settled on the eggs. To limit the contamination of the eggs, it was therefore suggested to the egg farming industry that extraction of eggs from the controlled LMS system should occur prior to the scraping and removal of fecal material.

2.5 References

Alaboudi, A.R., Hammed, D.A. and Ali, D.S. 1988. Microbial content of market eggs. *Indian Journal of Animal Sciences* 58, 768-770.

Andrews, W.H., Poelma, P.L. and Wilson, C.R. 1984. Isolation and identification of *Salmonella* species. *In*: Center for Food Safety and Nutrition. US FDA *Bacteriological Analytical Manual of the division of Microbiology*, (6th ed.) AOAC: 7.01-7.18: Arlington, Virginia.

Beever, R.E. and Bollard, E.G. 1970. The nature of the stimulation of fungal growth by potato extract. *Journal of General Microbiology* 60, 273-279.

Chang, Y.H. 2000. Prevalence of *Salmonella* spp in poultry broilers and shell eggs in Korea. *Journal of Food Protection* 63, 655-658.

Ching-Lee, M.R., Katz, A.R., Sasaki, D.M. and Minette, H.P. 1991. *Salmonella* egg survey in Hawaii: evidence for routine bacterial surveillance. *American Journal of Public Health*. 81, 764-766.

Davies, R.H., Nicholas, R.A.J., McLaren, I.M., Corkish, J.D., Lanning, D.G. and Wray, C. 1997. Bacteriological and serological investigation of persistent *Salmonella enteritidis* infection in an integrated poultry organization. *Veterinary Microbiology* 58, 277-293.

Donald, J. 1997. Planning for commercial layer expansion or renovation. *Poultry Housing Information Series* 701(8), 6-9.

Evans, M.R., Lane, W. and Ribeiro, D. 1998. *Salmonella enteritidis* PT6: Another egg-associated Salmonellosis? *Emerging Infectious Diseases* 4, 667-669.

Frazier, W.C. and Westhoff, D.C. 1988. Contamination, preservation, and spoilage of eggs. *In*: Frazier, W.C.and Westhoff, D.C. eds. *Food Microbiology* (pp. 255-267) (4th ed.) McGraw-Hill Book Company: New York.

Gentry, R.F. and Quarles, C.L. 1972. The measurement of bacterial contamination on eggshells. *Poultry Science* 51, 930-933.

Goto, S. and Enomoto, S. 1970. Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of Pseudomonas aeruginosa. *Japanese Journal of Microbiology* 4, 65-72.

Grijspeerdt, K. 2001. Modelling the penetration and growth of bacteria in eggs. *Food Control* 12, 7-11.

Hammack, T.S., Amaguana, R.M. and Andrews, W.H. 2001. Rappaport-Vassiladis medium for recovery of *Salmonella* spp from low microbial load foods: Collaborative study. *Journal of AOAC International* 84, 65-83.

Herbert, R.A. 1990. Methods for enumerating microorganisms and determining biomass in natural environments. *In*: Grigorova, R. and Norris, J.R. eds. *Methods of Microbiology* (pp. 1-39) Academic press: New York.

Humphrey, T.J. and Whitehead, A. 1992. Techniques for the isolation of *Salmonella* from eggs. *British Poultry Science* 33, 761-768.

International Commission on Microbiological Specifications for Food (ICMSF). 1980. Eggs and Egg Products *In*: Silliker, J.H., Elliot, A.C., F.L., Baird-Parker, J.H.B. Bryan, Christian, D.S., Clark, J.C., Olson, Jr. and Roberts, T.A. *Microbial Ecology of Foods* Vol II (pp 521-566). Academic Press: New York.

Jay, J.M. 2000. Miscellaneous food products. *In*: Jay, J.M. *Modern food microbiology* (6^{th} ed) (pp 163-175) Aspen publishers, Inc.: Maryland.

Kamel, B., Bond, C. and Diab, M. 1980. Egg quality as affected by storage and handling methods. *Journal of Food Quality* 3, 261-273.

Key, K. 1996. Egg-Based *Salmonella* on rise. *Health Letter on the CDC* 9, 2-4.

Kreager, K. 1999. Egg industry Initiatives to control *Salmonella*. *Poultry Bulletin* 10, 558-559.

Kurtzweil, P. 1998. Safer eggs: laying the groundwork. *FDA Consumer* 32,10-16.

Lee, J. 2000. New *Salmonella* finding – inter-bacterial communication! *Agricultural Research* 48(1), 10-11.

Manafi, M. and Kneifel, W. 1989. A combined chromogenic-fluorogenic medium for the simultaneous detection of Total Coliforms and *E. coli* in water. *Zentralblatt fur Hygiene* 189, 225-234.

Martley, F.G., Jayashankar, S.R. and Lawrence, R.C. 1970. An improved agar medium for the detection of proteolytic organisms in total bacterial counts. *Journal of Applied Bacteriology* 33, 363-370.

Mountney, G.J. 1989. *Poultry Products Technology*. (2nd ed.) Food Products Press: New York.

National Department of Agriculture of South Africa. 2001. The Resource Center; Directorate: Agricultural Information Services P/Bag X144 Pretoria Website: National Department of Agriculture of South Africa: Statistical Information: Trends in the Agricultural Sector 2001: Poultry: Egg Industry.[Online] http://www.nda.agric.za as viewed on November 3, 2002.

Nikanen, A. and Aalto, M. 1978. Comparison of selective media for coagulase-positive enterotoxigenic *Staphylococcus aureus*. *Applied and Environmental Microbiology* 35, 1233-1236.

Osborn, W.W. and Stokes, J.L. 1955. *Applied Microbiology* 3, 295.

Pienaar, A.C.E., Coetzee, L. and Bragg, R.R. 1995. A rapid method to determine bacterial contamination on hatching eggs. 2. Correlation of the optical-density measurements after incubation to bacterial counts on hatching eggs. *Onderstepoort Journal of Veterinary Research* 62, 25-33.

Radkowski, M. 2001. Occurrence of *Salmonella* sp. in consumption eggs in Poland. *International Journal of Food Microbiology* 64,189-191.

Schultze, G.E. and Fawcette, H.A. 1996. Prevalence of *Salmonella enteritidis* in poultry shell eggs in Arkansas[*]. *Southern Medical Journal* 89, 889.

South Africa. 1972. Foodstuffs, Cosmetics and Disinfectants Act, 1972 No. R.692 of 16 May 1997. Government Printers: Pretoria.

Tan, W. and Shelef, LA. 1999. Automated detection of *Salmonella sp.* in foods. *Journal of Microbial Methods*. 37(1), 87-91.

Todd, E.C.D. 1996. Risk assessment of use of cracked eggs in Canada. *International Journal of Food Microbiology* 30,125-143.

Trepka, M.J., Archer, J.R., Altekruse, S.F., Proctor, M.E. and Davis, J.P. 1999. An increase in sporadic and outbreak-associated *Salmonella Enteritidis* infections in Wisconsin: the role of eggs. *Journal of Infectious Diseases* 180, 1214-1219.

Vassiliadis, P. 1983. The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of *Salmonellas*: An overview. *Journal of Applied Bacteriology* 54, 69-76.

Venter, P., Lues, J.F.R. and Theron, H. 2003. The Quantification of bioaerosols in Automated Chicken Egg Production Plants. *Food Research International* (submitted for publication).

Zeidler, G. 1995. What makes microorganisms grow on eggs and in the egg room? (Part I) *Poultry Bulletin* 12, 363-365.

Chapter 3

THE QUANTIFICATION OF BIOAEROSOLS IN AUTOMATED CHICKEN EGG PRODUCTION PLANTS

Submitted for publication to: ***Food Research International***

3.1 Abstract

The quantity and composition of bioaerosols from automated chicken egg layer management system(s) (LMS) with a controlled internal environment (B) and without (A) were compared. Bioaerosols were collected using an air sampler in addition to the analysis of humidity, wind velocity, temperature and dust particle concentration. The average bioaerosol concentrations associated with the inside of the Type A LMS reached $\bar{x} = 1.1 \times 10^5$ cfu·m^{-3} with counts in the Type B LMS being $\bar{x} = 9.2 \times 10^4$ cfu·m^{-3}. In both cases the bacterial counts were significantly higher on the inside of the LMS than the outside. The Type A LMS showed yeast counts of $\bar{x} = 6.7 \times 10^1$ cfu·m^{-3} with none detectable in the Type B. Total culturable mold counts were $\bar{x} = 7.0 \times 10^2$ cfu·m^{-3} with significantly higher presumptive *Salmonella* spp counts ($\bar{x} = 6.6 \times 10^1$ cfu·m^{-3}) inside both LMS when compared to the outside. *E. coli* spp and total culturable Gram-negative counts were significantly higher in the Type B LMS at concentrations of $\bar{x} = 3.6 \times 10^1$ cfu·m^{-3}. These counts were significantly higher compared to the outside environment. The live birds were concluded to be the major source of fecal matter in bioaerosols in both LMSs with the fecal matter disposal systems contributing to the difference in bioaerosol composition. Modifications to the

operation protocols of both LMSs to limit the contamination of eggs by bio-aerosols were suggested.

3.2 Introduction

Modern methods for the mass production of chicken eggs are founded on advances towards increased production with minimum labour requirements. These advanced layer management system(s) (LMS) are usually confined structures densely stocked with egg-laying hens. While preceding systems relied upon manual labour, modern layer houses boast mechanical egg removal and fecal waste disposal systems. Egg production and chicken health are further maintained by regulating or simulating optimum environmental conditions, i.e. temperature and humidity, eliminating the control of biosecurity factors (rodents, insects etc.), breed and health of the introduced chicken as well as fodder composition as factors affecting egg quality and production (Salatin, 1993; Ensimger, 1992).

In these densely populated and enclosed buildings microorganisms originating from fecal matter and feeding material, if not controlled, accumulate and are easily aerosolized. Resulting viable airborne contaminants (bioaerosols) could be solid, liquid, borne by other particles or suspended in liquid droplets. They may contain bacteria, bacterial spores, fungi or fungal spores, antigens, toxins, viruses, plant pollens and fecal matter. These bioaerosols are all possible product contaminants and may, in addition, affect employee health (American Conference of Governmental Industrial Hygienists, 1989; Donham *et al.*, 1986).

Organisms regularly associated with bioaerosols originating from densely populated environments such as swine houses have been shown to reach levels of 10^5-10^7 cfu·m^{-3} (Crook *et al.*, 1991; Cormier *et al.*, 1990; Donham *et al.*, 1986; Clark *et al.*, 1983). These aerosolized populations include fungi at levels of up to 10^4 cfu·m^{-3} as well as bacteria, which are dominated by Gram-positive species. In poultry-slaughtering plants the identified bacteria within bioaerosols follow a similar trend and were also reported to be predominantly Gram-positive. This group included *Bacillus* spp and *Staphylococcus* spp whereas the Gram-negative bacteria included genera such as *Acinetobacter*, *Proteus* and *Escherichia coli* (Lenhart *et al.*, 1982). According to Lenhart *et al.* (1982) the major sources of bioaerosol within these plants are the live birds. The authors further noted that 62 % of the isolated bacilli were *E. coli*, a bacterium of fecal origin.

Other sources of bioaerosols that are not related to the chickens could also occur in egg production plants. These include contaminants from waste treatment systems, building maintenance, bacterial or fungal growth in the environment. Facility workers may also be a source of bioaerosol contaminants, which can be carried to work on clothes or skin. Fecal contaminants of human origin may also be introduced into the workplace as a result of poor personal hygiene practices (Lutgring *et al.*, 1997). Another major impacting factor on the aerosolizing and distribution of airborne microorganisms is ventilation systems. The ventilation system directly affects factors such as relative humidity and temperature, further influencing the viability of bioaerosols present, the time spent airborne and the size of carrier droplets - all of which determine the bioaerosol composition and contamination levels of exposed food products (Heldman, 1974).

Given the differences in LMS facility design (controlled environment or open air) and variations in facility ventilation, the effects of these factors on the concentration, distribution and composition of bioaerosols are not completely understood when considering the quality of the eggs produced. In the South African chicken egg industry, information on the dispersion of bioaerosols in the different kinds of LMS is lacking, leading to considerable expenditure on facility design without knowledge of the effect on bioaerosol dispersion. The aim of this study was to determine the levels and distribution of viable airborne microorganisms in two commonly used systems (controlled and uncontrolled) and further to evaluate the effects of different environmental factors on the bioaerosol composition. Finally, the two LMS systems were compared regarding their ability to control the respective bioaerosol compositions.

3.3 Materials and methods

3.3.1 Sampling

A chicken egg farm boasting two types of LMS, one with a controlled internal environment (producing ± 20 000 eggs·day^{-1}) and another with an uncontrolled internal environment (producing ± 20 000 eggs·day^{-1}) was selected for this study. Bioaerosol samples were collected 1.5m above the floor of the parallel walkways between the high-rise battery sets of both LMS as well as from the outside environment. Temperature, relative humidity, wind velocity and dust particle concentration were measured at the same height as the bioaerosols using direct reading instruments (temperature: Area Heat Stress Monitor – Questemp, SA; relative humidity: LMS Automated Humidity Meter; wind velocity: anemometer - Airflow

Instrumentation, SA; airborne particle concentration: handheld aerosol monitor - PPM Enterprises, Inc., SA).

Bioaerosol sampling and analysis

Microbial bioaerosols were measured by impaction on agar plates using a single stage SAS Super 90 air sampler (Thorne *et al.*, 1992; Heedrich, *et al.*, 1991; Cormier, 1990; Donham *et al.*, 1989; Haglind and Rylander, 1987; Donham *et al.*, 1986; Clark *et al.*, 1983). The air sampler was pre-calibrated at 28.3 $l \cdot min^{-1}$ before sampling and all removable components of the air sampler were pre-autoclaved as well as disinfected with 70% ethanol between each sampling run. The air sampler operates by directly collecting airborne microbes onto 55mm RODAC plates containing Plate Count Agar (PCA), MacConkey Agar (MCA), Potato Dextrose Agar – pH=4 (PDA) and Chromocult Coliform Agar™ (CCA) respectively at each sampling location. These media were used for the selective cultivation and enumeration of the total aerobic plate counts (APC) (Martley *et al*, 1970), total viable Gram-negative bacteria (Atlas and Parks, 1993), total viable yeast and moulds (Beever and Bollard, 1970), total *Escherichia coli* and presumptive *Salmonella* spp (Kilian and Bülow, 1976; Framton *et al.*, 1988; Manafi and Kneifel, 1989). All evaluations were performed twice in addition to field blank samples (sampler not operating) at all sampling locations. The plates were incubated at 37°C for 48 hours for the CCA and 25°C for 24-48 hours for the PCA, MCA and PDA. Colonies were counted using a Gerber colony counter. The positive-hole method and the period (seconds) sampled was applied to the results from the air sampler for corrections of microbial coincidence (Macher, 1989).

3.3.2 Environmental factors

The temperature, wind velocity and relative humidity were recorded from 10:30 to 16:00 for both sampling sites using the previously mentioned direct reading instruments. All readings were recorded in duplicate.

3.3.3 Statistical analysis

Data reported are the means of duplicate repetitions at each sampling point. Significance was set at $P \leq 0.05$.

3.4 Results and discussion

3.4.1 LMS characteristics

The first type of LMS (A) (Fig. 3.1A) was a dual level structure characterised by a construction with several rows of high-rise hen batteries (top level) equipped with an automated egg removal mechanism. Metal-sheet roofs and walls further enclosed the upper level of the LMS. The fecal matter disposal system featured a central opening in the floor through which the matter automatically dropped to an open-air lower level, and was removed annually. Except for extractor fans at the one end of the upper level and air vents in the roof, no further ventilation and environmental control systems were present. An enclosed (smooth brick wall) and mechanically-ventilated egg grading, quality control and packaging unit was situated adjacent to the LMS. Only one section of this unit served the adjacent LMS (Fig. 3.1A, [10]) whilst the remaining section (Fig. 3.1A, [11]) served the Type B LMS.

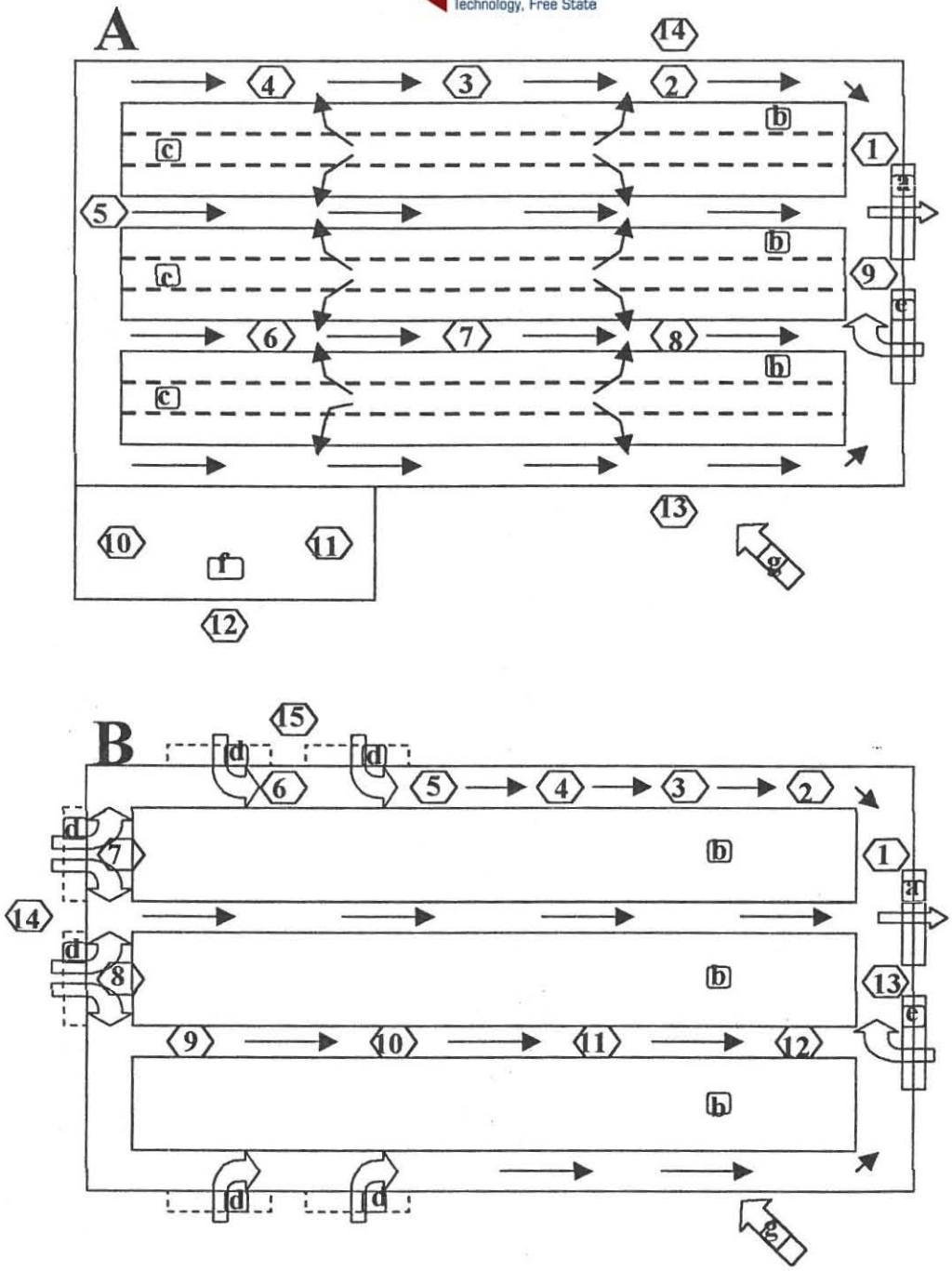


Fig. 3.1 Facility design, direction of internal airflow (all arrows), extraction fans present (a-operative, e-non-operative), high-rise hen battery sets (b), floor opening to lower fecal catchment level (c), inlet water cooled air filters (d), egg grading – quality control – packaging unit (f), external wind direction (g) of both a layer management system with an uncontrolled internal environment (A) and a system with a controlled internal environment (B). All numbers represent sampling locations.

The second type of LMS (B) (Fig. 3.1B) was similar to the first except that it was a single level structure enclosed with smooth brick walls boasting an automated fecal matter removal mechanism. This system included a conveyer belt below each hen battery set, which moved the fecal matter to the one end of the LMS (each morning) from where it was loaded onto a truck. A fully automated environmental control system in this LMS regulated temperature and humidity by means of water-cooled filters located in the walls at one end and extraction fans at the opposite.

Both systems housed chickens of a similar age at similar cage density. They utilised the same type of cages (four levels in the high-rise) and biosecurity plan. The two manure removal mechanisms (though different) could be classified as dry removal and not flush systems.

3.4.2 Environmental factors

The wind velocity, temperature and airborne particle concentration at each sampling location are shown in Fig. 3.2. No significant difference in relative humidity was noticed over the various sampling periods. In both types of LMS the wind direction was towards the extraction fans originating in Type A from the lower fecal catchment level and in Type B from the imbedded wall filters. No significant differences were evident between environmental factors of both LMS A and B – wind velocity inside (P=0.197), outside (P=0.322); airborne particles inside (P=0.129), outside (P=0.213); temperature inside (P=0.057), outside (P=0.291).

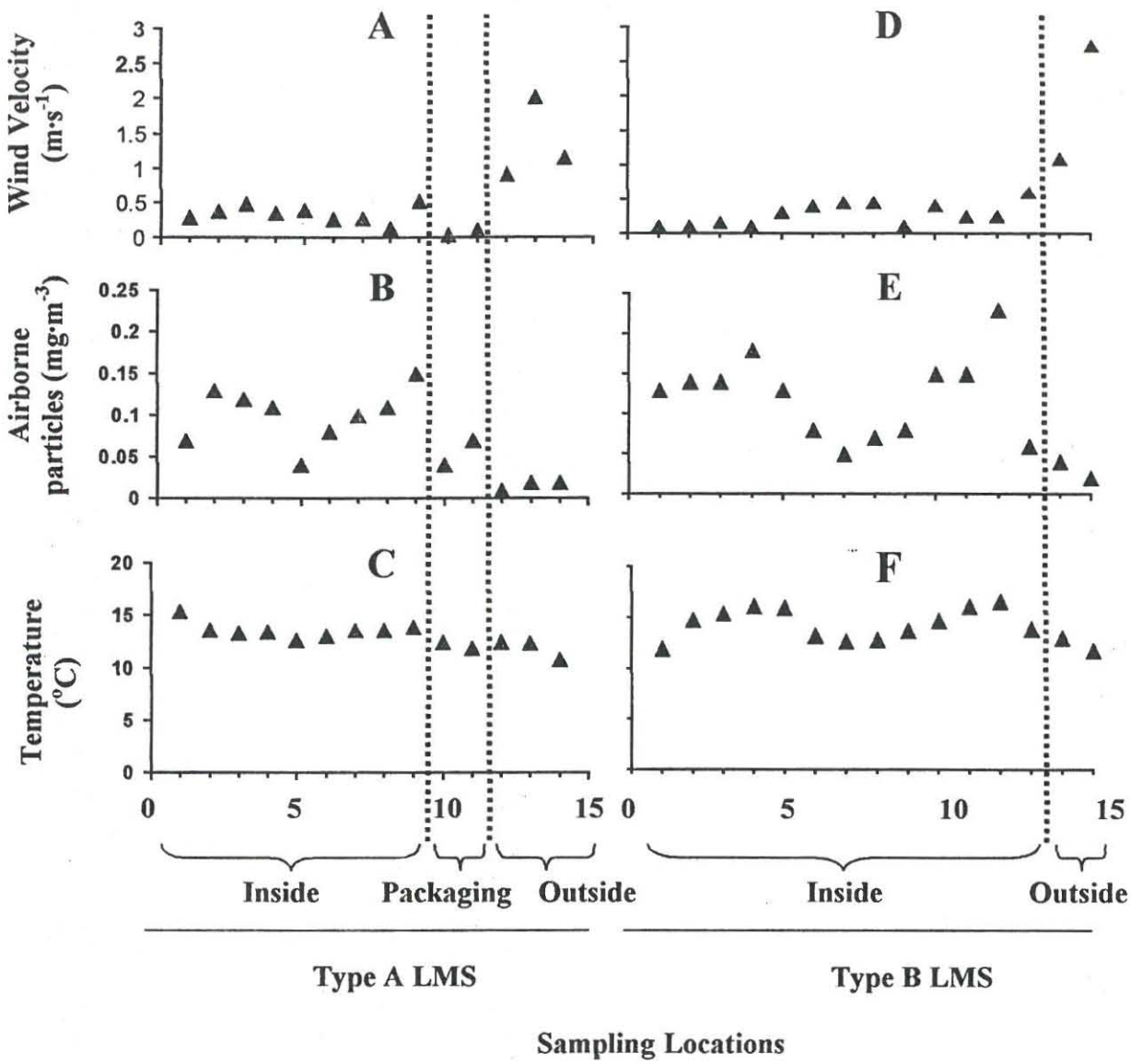


Fig. 3.2 The levels of different environmental factors encountered in and outside a layer management system with an uncontrolled inner environment (A-C) and one with a controlled inner environment (D-F).

Culturable airborne microorganisms – Type A LMS

The average bioaerosol concentrations associated with the Type A LMS are shown in Fig. 3.3. The total APC ranged from $8.1 \times 10^3 - 3.6 \times 10^5$ cfu·m^{-3} ($\bar{x} = 1.1 \times 10^5$ cfu·m^{-3}) (Fig. 3.3A) amongst sampling points 1-9. These concentrations were significantly higher ($P = 0.026$) than those of the outside environment ($7.0 \times 10^2 - 3.9 \times 10^3$ cfu·m^{-3}; $\bar{x} = 9.2 \times 10^4$ cfu·m^{-3}); sampling points 12-14.

Culturable yeast collected from the Type A LMS ranged from $0 - 2.0 \times 10^2$ cfu·m^{-3} ($\bar{x} = 6.7 \times 10^1$ cfu·m^{-3}) and this did not differ markedly from samples collected from the outside environment (Fig. 3.3B). This was also the case with culturable moulds ranging from $0 - 4.3 \times 10^3$ cfu·m^{-3} ($\bar{x} = 7.0 \times 10^2$ cfu·m^{-3}) (Fig. 3.3C). Presumptive *Salmonella* spp counts, though low, ranged from $0 - 1.0 \times 10^2$ cfu·m^{-3} ($\bar{x} = 6.6 \times 10^1$ cfu·m^{-3}) on the inside to $0 - 3.3 \times 10^1$ cfu·m^{-3} ($\bar{x} = 1.1 \times 10^1$ cfu·m^{-3}) on the outside ($P = 0.006$) (Fig. 3.3D). *E. coli* counts were only observed in one sample at a concentration of 6.6×10^1 cfu·m^{-3} on the inside of LMS (A) with similar counts on the outside (Fig. 3.3E). The adjacent egg grading, quality control and packaging unit (sampling points 10-11) did not differ significantly from the inside of the Type A LMS. The total culturable Gram-negative bacterial levels ranged from $0–6.6 \times 10^1$ cfu·m^{-3} ($\bar{x} = 1.5 \times 10^1$ cfu·m^{-3}) inside the LMS (Fig. 3.3F) with similar results in the egg grading, quality control and packaging unit as well as in the outside environment.

Culturable airborne microorganisms – Type B LMS

Fig. 3.3 shows the average bioaerosol concentrations inside as well as outside the Type B LMS. The total APC bacteria obtained from inside the Type B LMS

(sampling points 1-13) ranged from $9.0 \times 10^2 - 1.6 \times 10^5$ cfu·m^{-3} ($\bar{x} = 9.2 \times 10^4$ cfu·m^{-3}) – and are significantly ($P = 0.0002$) higher compared to the outside environment ($1.1 \times 10^3 - 1.4 \times 10^3$ cfu·m^{-3}; $\bar{x} = 1.3 \times 10^3$ cfu·m^{-3}) sampling points 14-15. No culturable yeast was detected inside the Type B LMS. Culturable moulds ranged from $0 - 3.0 \times 10^2$ cfu·m^{-3} ($\bar{x} = 6.2 \times 10^1$ cfu·m^{-3}) showing a significant ($P = 0.0002$) difference compared to the outside (Fig. 3.3C). Culturable presumptive *Salmonella* concentrations ranged from $0 - 2.0 \times 10^2$ cfu·m^{-3} ($\bar{x} = 7.4 \times 10^1$ cfu·m^{-3}) on the inside with none detectable on the outside (Fig. 3.3D). Similarly, *E. coli* counts ranged from $0 - 1.0 \times 10^2$ cfu·m^{-3} ($\bar{x} = 3.6 \times 10^1$ cfu·m^{-3}) on the inside, with none detectable on the outside (Fig. 3.3E). The total culturable Gram-negative bacterial levels ranged from $0 - 1.3 \times 10^2$ cfu·m^{-3} ($\bar{x} = 4.8 \times 10^1$ cfu·m^{-3}) inside the LMS compared to the outside environment were no Gram-negative bacteria were detected (Fig. 3.3F).

Little difference was evident in the relative humidity between the two types of LMS and their separate effects on the bioaerosol concentration were therefore negligible. The wind velocity inside the Type A LMS was on average below 0.5 m·s^{-1}, with the exception of sampling point 9 where the extractor fan located closest to the sampling point, which was inoperative at the time of sampling and instead acted as a window for incoming air from the outside environment. Little dispersion and dilution, however, of airborne contaminants occurred by the low wind force from the lower level towards the upper as well as throughout this system (Fig. 3.2A). This limited dilution effect is evident from the elevated concentration of airborne particles between the high-rise battery sets (Fig. 3.2B, samples 2-4 and 6-8). The lower airborne particle counts detected within the egg grading, quality control and packaging area as

well as outside signifies the chickens as the major source of these particles. These results seem to support the comments of Lenhart *et. al.* (1982), who identified the live birds in poultry slaughtering plants as the major source of bioaerosols.

Similar levels of wind velocity were detected in the Type B LMS (Fig. 3.2D), with the exception of sampling sites 5 - 8, which were taken in close proximity to the inlet filters. Samples collected amongst the high-rise egg batteries of this LMS showed low wind velocities, again with little dispersion and dilution of airborne contaminants. The elevated wind velocity noted at sampling points 5-8 (Fig. 3.2D) together with the lower temperatures and airborne particle concentration (Fig. 3.2E, F) indicated a constant fresh feed of outside air into the LMS. Elevated airborne particle concentrations amongst the high-rise battery sets of the LMS again implicate the birds as the major source of bioaerosols.

Culturable airborne microorganisms

The total APC were on average lower in both types A and B LMS, than have previously been reported (Lenhart *et al.*, 1982; Clark *et al.*, 1983; Donham *et al.*, 1986; Cormier, 1990; Crook *et al.*, 1991). The total APC, presented in Fig. 3.3A, showed similar results throughout the sampling spectrum with only the samples collected from the outside environment presenting lower levels. The total APC in the outside environment were significantly lower than the inside of both the LMS and significant contamination of the eggs (whilst in the LMS) by this source is thus improbable.

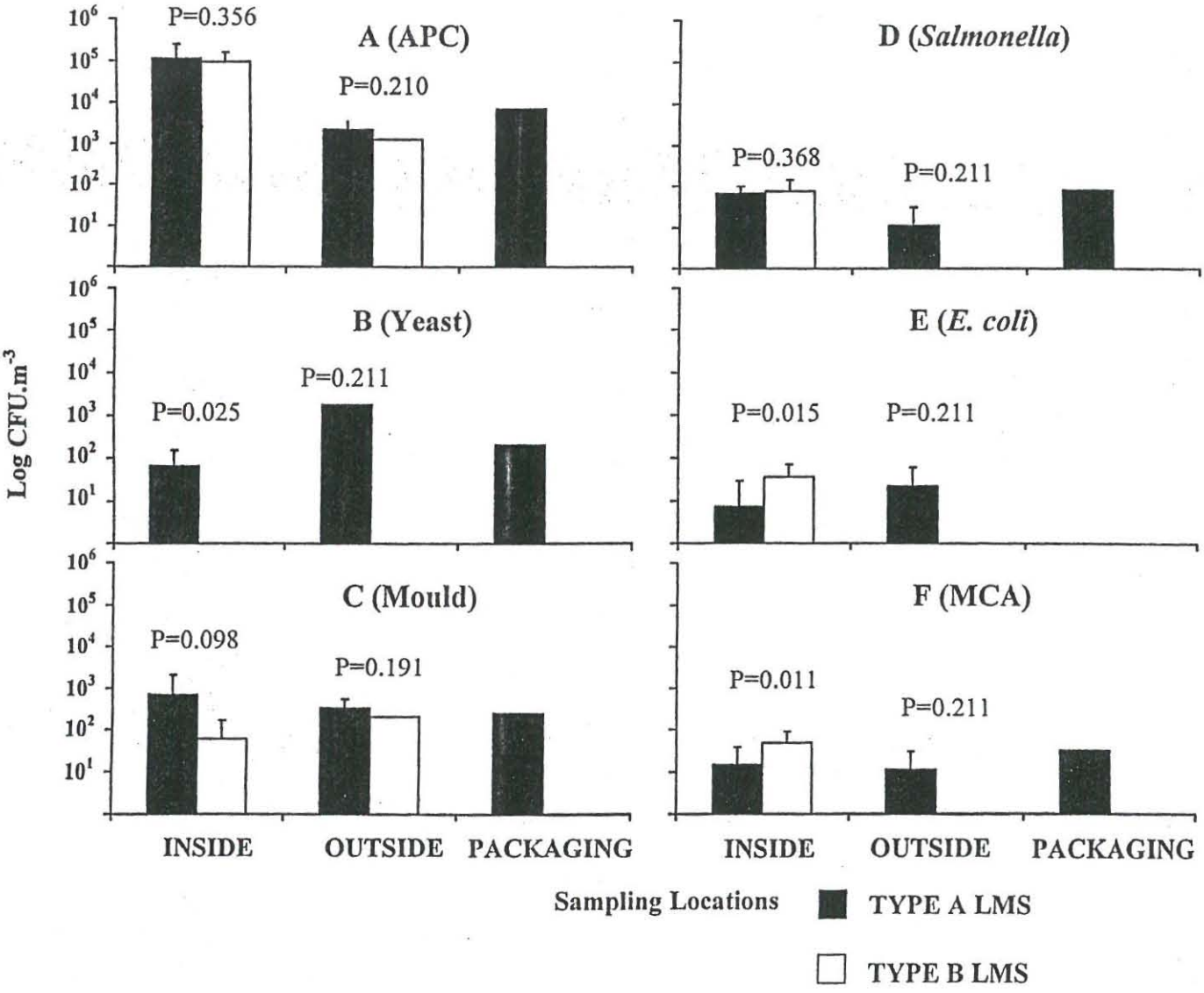


Fig. 3.3 The average culturable airborne microorganism concentrations in both a layer management system with an uncontrolled inner environment (Type A LMS) and one with a controlled inner environment (Type B LMS). Bioaerosol concentrations A: Total APC; B: Total viable yeast; C: Total viable moulds; D: Total viable presumptive *Salmonella* spp counts; E: Total viable *Escherichia coli* counts; F: Total viable Gram-negative bacterial counts.

3.4.3 Comparison between LMS A and LMS B

When comparing the indoor bioaerosol composition of the two types of LMS designs, distinctive differences were noted. Aerosolized yeast was only detected in the Type A LMS whereas moulds were present in the bioaerosol of both types of LMS ($P = 0.09$). Likewise, presumptive *Salmonella* counts revealed a high degree of similarity between the two LMS whereas *Escherichia coli* counts were, on the other hand, significantly higher in the Type B LMS ($P = 0.014$). This difference is probably ascribable to the bird faeces, which is scraped each morning in the Type B LMS producing higher concentrations of aerosolized particles of fecal origin (Theron *et al.*, 2003). The total culturable Gram-negative bacterial counts supported this observation in that the Type B LMS presented significantly higher counts of these microbiota ($P = 0.011$). Relatively low counts of viable Gram-negative bacteria were detected when compared with the results obtained from the CCA. This low sensitivity supports reports by Chang *et al.* (2001), who noted relatively low counts of Gram-negative bacteria compared to previous reports (Crook *et al.*, 1991; Cormier, 1990; Donham *et al.*, 1986; Clark *et al.*, 1983).

In conclusion, airborne culturable bacterial levels in both types LMS evaluated were lower than those cited in previous studies. Structural differences between the two types of LMS evaluated might influence the quality of the eggs produced as well as the quality of air inhaled by workers. The major benefit of an automated fecal matter disposal system, compared to the open air lower catchment level, is the decrease in labour requirements; however, in this study it was found to be a prominent factor in the generation of bio-aerosols. In order to limit the effect of this system on the quality

of the eggs the LMS should be set to extract already laid eggs before scraping the fecal matter from the conveyer belts. Although the control of environmental conditions i.e. temperature and relative humidity, are said to increase the levels of egg production, no significant effect on the concentration of bioaerosols was noted. The direct effect of airborne contamination on the quality of eggs produced in these types of LMS has however not been evaluated. Further research to establish the direct effect of airborne contamination on egg shelf-life and quality is thus required.

3.5 References

American Conference of Governmental Industrial Hygienists. 1989. *Guidelines for the assessment of bioaerosols in the indoor environment*. ACGIH, Cincinnati, Ohio.

Atlas, R.M. and Parks , L.C. 1993. *Handbook of microbiological media.* CRC Press, Inc.: London.

Beever, R. E., and Bollard, E. G. 1970. The nature of the stimulation of fungal growth by potato extract. *Journal of General Microbiology* 60, 273-279.

Chang, C. W., Chung, H., Huang, C. F. and Su, H. J. J. 2001. Exposure of workers to airborne microorganisms in open-air swine houses. *Applied and Environmental Microbiology* 67, 155-161.

Clark, S., Rylander, R. and Larsson, L. 1983. Airborne bacteria, endotoxins and fungi in dust in poultry and swine buildings. *American Industrial Hygiene Association Journal* 44, 537-541.

Cormier, Y., Tremblay,G., Meriaux, A. Brochu, G. and Lavoie, J. 1990. Airborne microbial contents in two types of swine confinement buildings in Quebec. *American Industrial Hygiene Association Journal* 51, 304-309.

Crook, B., Robertson, J. F., Travers Glass, S. A., Botheroyd, E. M., Lacey, J. and Topping, M.D. 1991. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. *American Industrial Hygiene Association Journal* 52, 271-279.

Donham, K. J., Haglind, P., Peterson, Y., Rylander, R. and Belin, L. 1989. Environmental and health studies of farm workers in Swedish swine confinement buildings. *British Journal of Industrial Medicine* 46, 31-37.

Donham, K. J., Popendorf, W., Palmgren, U. and Larsson, L. 1986. Characterization of dusts collected from swine confinement buildings. *American Journal of Industrial Medicine* 10, 294-297.

Ensimger, M. E. 1992. *Poultry science* (3rd ed.). Interstate Publishers, Inc.: Danville, I.L.

Frampton, E. W., Restaino, L. and Blaszko, L. 1988. Evaluation of β-glucuronidase substrate 5-bromo-4-chloro-3indol-β-D-glucuronide (X-GLUC) in a 24 hour direct plating method for *Escherichia coli*. *Journal of Food Protection* 51, 402-404.

Haglind, P. and Rylander, R. 1987. Occupational exposure and lung measurements among workers in swine confinement buildings. *Journal of Occupational Medicine* 29, 904-907.

Heedreich, D., Brouwer, R., Biersteker, K. and Boleij, J. S. M. 1991. Relationship of airborne endotoxins and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *International Archives of Occupational and Environmental Health* 62, 595-601.

Heldman, D. R. 1974. Factors influencing airborne contamination of foods: a review. *Journal of Food Science* 39:962-968.

Kilian, M., and Bülow, P. 1976. Rapid diagnosis of Enterobacteriaceae. *ACTA Pathologica et Microbiologica Scandinavica Section B-Microbiology* 84, 245-251.

Lenhart, S. W., Olenchock, S. A. and Cole, E. C. 1982. Viable sampling for airborne bacteria in a poultry processing plant. *Journal of Toxicology and Environmental Health* 10, 613-619.

Lutgring, K. R., Linton, R. H., Zimmerman, N. J., Peugh, M. and Heber, A. J. 1997. Distribution and Quantification of bioaerosols in poultry-slaughtering plants. *Journal of Food Protection* 60, 804-810.

Macher, J. 1989. Positive hole correction of multiple-jet impactors for collecting viable microorganisms. *American Industrial Hygiene Association Journal* 50, 561-656.

Manafi, M., and Kneifel, W. 1989. A combined chromogenic-fluorogenic medium for the simultaneous detection of Total Coliforms and *E. coli* in water. *Zentralablatt fur Hygiene* 189, 225-234.

Martley, F. G., Jayashankar, S. R., and Lawrence, R. C. 1970. An improved agar medium for the detection of proteolytic organisms in total bacterial counts. *Journal of Applied Bacteriology* 33, 363-370.

Salatin, J. 1993. *Pastured poultry profits*. Polyface: Swoope, V.A.

Theron, H., Venter, P. and Lues, J.F.R. 2003. Microbial hazard identification of chicken eggs produced in high-rise layer systems. *Food Control* (submitted for publication).

Thorne, P. S., Kiekhaefer, M. S., Whitten, P. and Donham, K. J. 1992. Comparison of bioaerosol sampling methods in barns housing swine. *Applied and Environmental Microbiology* 58, 2543-2551.

Chapter 4

BACTERIAL GROWTH ON CHICKEN EGGS IN VARIOUS STORAGE ENVIRONMENTS

Submitted for publication to ***Food Research International***

4.1 Abstract

This study was conducted to determine the effect of various storage environments on bacterial growth patterns, shelf-life and safety of eggs. Two hundred and ten chicken egg samples were randomly collected from a layer management system that employs a fully automated environmental control system. Eggs were subjected to temperature shocks to simulate various transport and storage conditions. When monitored over a period of 17 days, it was found that temperature shocks influenced the counts of microbial populations both on the eggshells and in the egg contents. Results indicated that 4-hour and 6-hour low temperature shocks were the most effective in limiting microbial growth. Much higher organism counts were found in both the egg contents and on the eggshells, when subjected to high temperature shocks. In addition, results showed that temperature abuse could lower egg quality and shorten the shlef-life of the product. To improve shelf-live, it was recommended to the egg-producing industry that the 4-hour shock at 4°C should be employed during the storage and transport of eggs.

4.2 Introduction

The egg is a highly perishable food product, which could lose its quality rapidly during the period between collection and consumption. The shelf-life of eggs is further influenced by both extrinsic (such as environmental conditions) and intrinsic factors (such as nutrients and water activity) (Kamel *et al.*, 1980). In the European Community and the United States eggs are generally stored between 8°C and 16°C, whereas in South Africa eggs are generally stored at room temperature (Schoeni *et al.*, 1995).

On farms where eggs are produced, the source of bacterial contaminants has been shown to be the environment as well as the chickens (Todd, 1996). Literature furthermore suggests that aerosolization of fecal particles with associated microorganisms could settle on eggs (Venter *et al.*, 2003). Wang and Slavik (1998) further noted that bacteria on the surface of the shell are able to pass through the pores of the shell to contaminate the interior of the egg, even though the eggshell has physical barriers, and the albumen in the egg white has characteristics to prevent microbial growth (Frazier and Westhoff, 1988). Schoeni *et al.* (1995) emphasised the need to remove any fecal contamination rapidly in order to reduce the risk of microbial penetration into the contents of the egg.

In recent years much attention has been given to the role chicken eggs play in the transmission of bacteria such as *Salmonella* in Europe (Lee, 2000), Canada (Todd, 1996), America (Trepka *et al.*, 1999; Schultze and Fawcette, 1996; Ching-Lee *et al.*, 1991) and countries like Kuwait (Kamel *et al.*, 1980), Korea (Chang, 2000) and Poland (Radkowski, 2001). According to Lee (2000) the occurrences of egg-related

food poisoning have increased four-fold in the United States and forty-fold in Europe. In South Africa the first recorded poultry-associated outbreak of *Salmonella entertidis* occurred in 1991 in the Western Cape Province (Maré *et al.*, 2000).

At present, quality control and proper processing facilities are in many instances not up to standard in developing countries while legislation to govern the egg industry is lacking in South Africa. Therefore, a high percentage of eggs deteriorate and eventually go to waste.

The aim of this study was to examine the effects of various storage and transport conditions on the bacterial growth associated with chicken eggs that are subjected to adverse temperatures during transportation. Six microbial groups associated with eggs were identified as potential hazards in or on eggs and were quantified by using the streak plate method on selective agars. The results obtained provided information on the improvement of the shelf-life of chicken eggs by controlling transportation and storage conditions.

4.3 Materials and methods

4.3.1 Sampling

Two hundred and ten chicken eggs were randomly collected from a layer management system that houses about 20 000 hens and employs a fully automated environmental control system. This system regulates the temperature and humidity by means of water-cooled filters embedded in the walls at the one end and extraction fans at the other. The system is further equipped with an automated fecal removal mechanism via conveyer belts set up below each cage set.

4.3.2 Transport and storage simulations

With the egg production site situated ± 50 km from the retail outlets, both low (4°C) and high (37°C) transport temperature conditions were used in the investigation of the influence of time and temperature on the microbial quality change of the eggs during transportation. The respective simulations included: a) *Simulation 1*: 3 egg batches (25 eggs / batch) were subjected to a 4°C transport simulation over 3 time intervals (2, 4 and 6 hours respectively), followed by 25°C incubation at a constant humidity for the duration of the study (17 days), simulating storage at room temperature; b) *Simulation 2*: a similar experimental design to *Simulation 1* except that the simulated transport temperature was 37°C; c) *Simulation 3 (low temperature control)*: 8°C refrigeration temperatures for the duration of the study (17 days); d) *Simulation 4 (high temperature control)*: incubation for the duration of the study (17 days) at 25°C.

For the quantification of microorganisms on the shell and inside the eggs, a sample consisting of five eggs was randomly drawn from each simulation batch at regular intervals, namely at 0 and 3, 5, 8, 10 and 17 days after treatment.

Microbial contamination on the eggshell

A modification of the egg washing procedure used by Pienaar *et al.* (1995), Alaboudi *et al.* (1988) and Gentry and Quarles (1972) was used to isolate the microorganisms from the eggs. Each egg was gently washed in sterile 50 ml Nutrient Broth in a sterile plastic bag by rubbing the surface of the eggs, through the plastic bag, for 30 seconds (Pienaar *et al.*, 1995). The broths from five egg washings were combined and analysed (Alaboudi *et al.*, 1988).

Analysis of the egg contents

After washing, the eggshells were sterilised by briefly submerging them in alcohol (99%), followed by drying in a sterile cabinet. The contents of five eggs were combined by aseptically cracking open the eggs and emptying the contents into 500 ml sterile Nutrient Broth, after which the mixture was shaken until evenly mixed (Schultze and Fawcette, 1996).

For both the eggshells and the egg contents, serial dilutions were prepared with the use of a saline solution (Biolab-SA). The spread plate method was employed to quantify the various microbial groups (Herbert, 1990) using the following agars: Aerobic Plate Counts (APC, MERCK, Martley *et al.*, 1970) followed by incubation at 25°C for 48 hours for the enumeration of the total aerobic plate counts (APC); Cetrimide Agar with added glycerol (MERCK, Goto and Enomoto, 1970) followed by incubation at 25°C for 18-48 hours for *Pseudomonas* spp; Baird-Parker Agar (MERCK, Nikanen and Aalto, 1978) followed by incubation at 36°C for 48 hours for *Staphylococcus* spp and Chromocult Coliform Agar (MERCK, Manafi and Kneifel, 1989) followed by incubation at 36°C for 48 hours for Total coliforms and *Escherichia coli*.

For *Salmonella* spp 10 ml of the Nutrient Broth from the egg mixture was transferred to 100 ml of Rappaport Vassiliadis medium (SCHARLAU, Vassiliadis 1983, Hammack, Amaguana and Andrews, 2001) followed by incubation at 42°C for 24 hours. An aliquot of the sample was consequently used to prepare a spread plate on Brilliant Green Agar plates (BGA) (SCHARLAU, Osborn and Stokes, 1955) and incubated for 24-48 hours at 42°C.

4.3.3 Data analysis

Data reported are means of duplicate repetitions for each sample. All 3-dimensional surface plots of microbial growth and 2-dimensional plots for the evaluation of the influence of temperature fluctuation on microbial growth were generated with SPSS Tablecurve 3D (version 4) for Windows. For normally distributed data MINITAB (version 12.1) for Windows was used for analysis of significance (P) and correlation (r), amongst other factors. Significance limit was set at $P \leq 0.05$.

4.4 Results

4.4.1 Bacterial growth and interactions in the egg contents

High and Low Temperature Control (Treatments 1 and 2) (Figs 4.1A-4.6F)

Although organism growth was limited in the egg contents during treatment 1 (25°C), marked growth was found for Total coliforms (Fig. 4.3C) after a prolonged lag phase (8 days). Limited growth at 25°C was evident with *Salmonella* spp (Fig. 4.2B). The APC correlated strongly with both Total coliforms (0.991) and *Salmonella* spp (0.990). During treatment 2 the low temperature (8°C) treatment also resulted in a prolonged lag phase of 8 days (as observed in treatment 1), followed by exponential growth for Total coliforms (Fig. 4.3C), *Pseudomonas* spp (Fig. 4.4D) and APC (Fig. 4.6F) in the egg contents. These organisms followed a K strategic growth pattern exhibiting an extended lag phase (Atlas and Bartha, 1993), giving the organisms time to adapt to the antimicrobial activity known to occur in eggs (Frazier and Westhoff, 1988). In addition the APC correlated strongly with *Pseudomonas* spp (0.996) and Total coliforms (0.999).

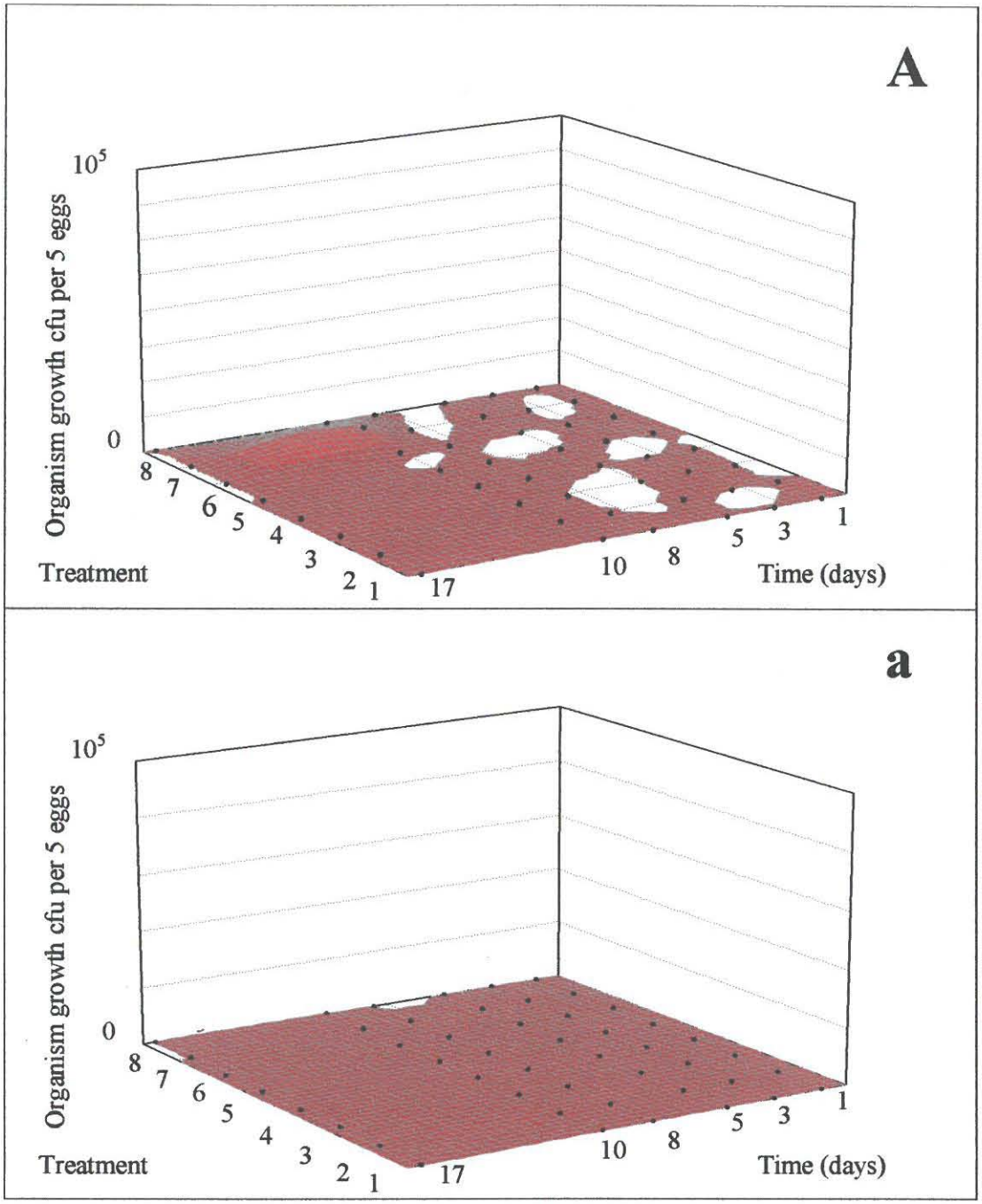


Fig. 4.1 Results of test for *E. coli* in the egg contents (A) and on the eggshell (a) during treatments 1-8 for the duration of 17 days.

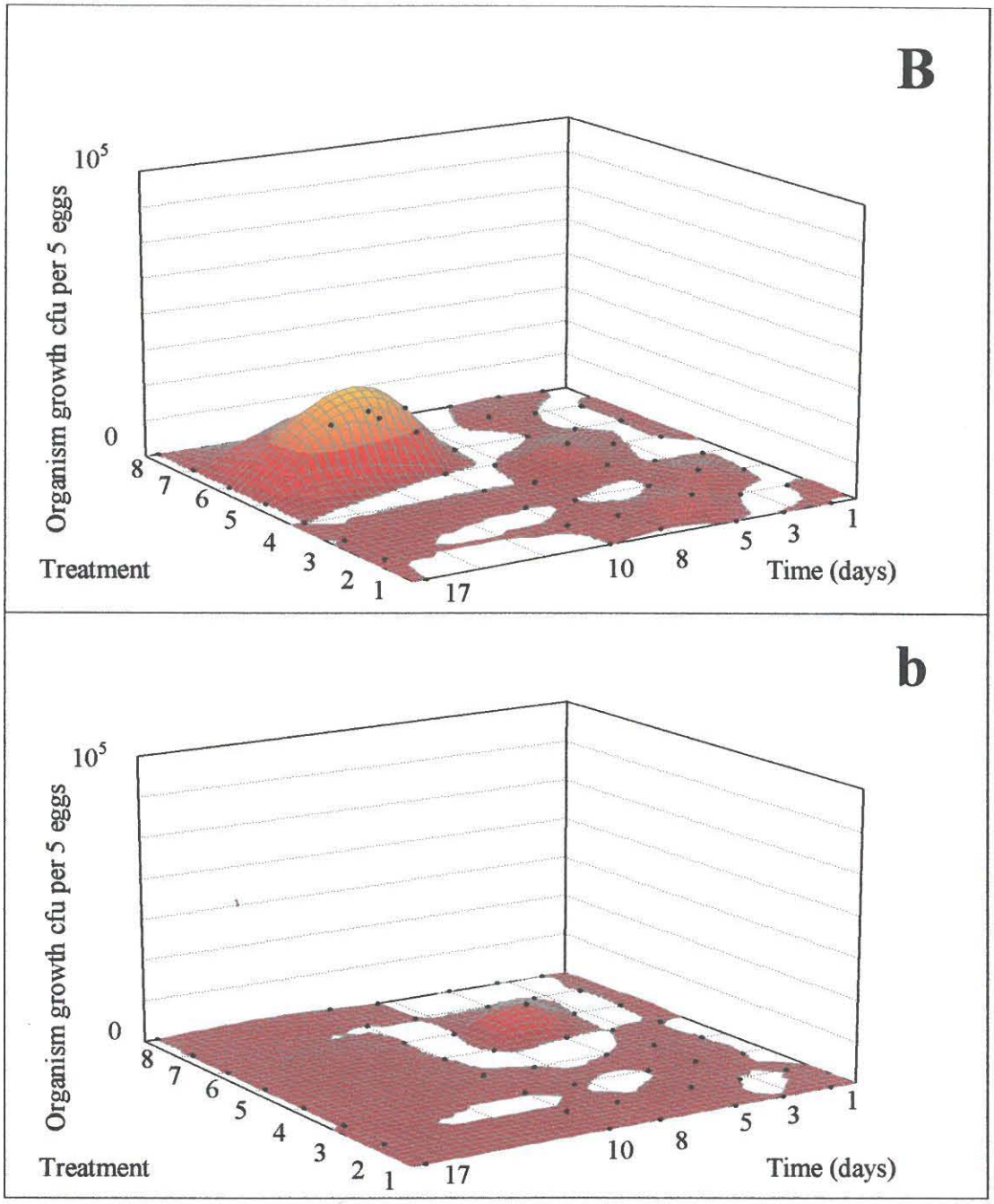


Fig. 4.2 Results of test for *Salmonella* spp in the egg contents (B) and on the eggshell (b) during treatments 1-8 for the duration of 17 days.

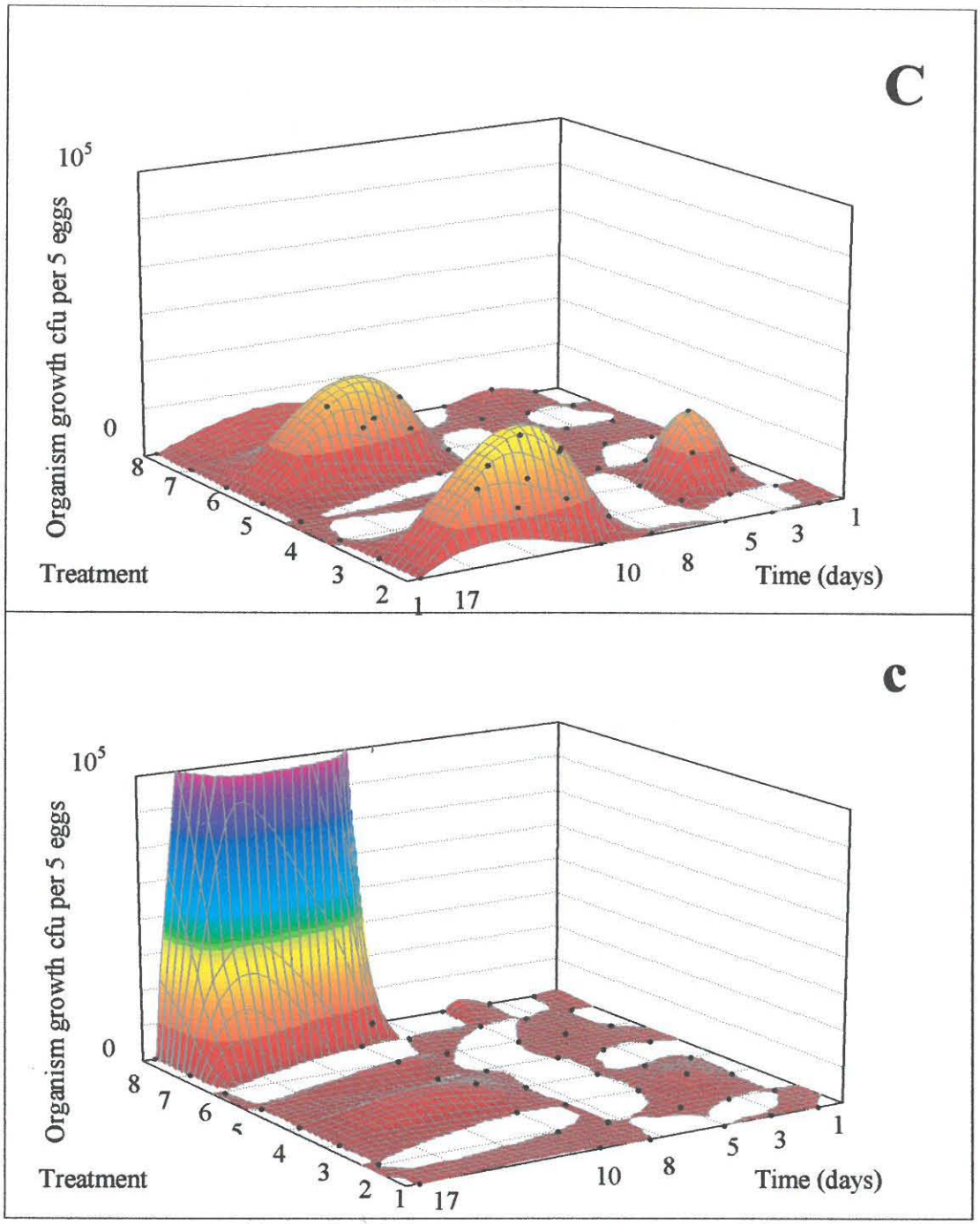


Fig. 4.3 Results of test for Total coliforms in the egg contents (C) and on the eggshell (c) during treatments 1-8 for the duration of 17 days.

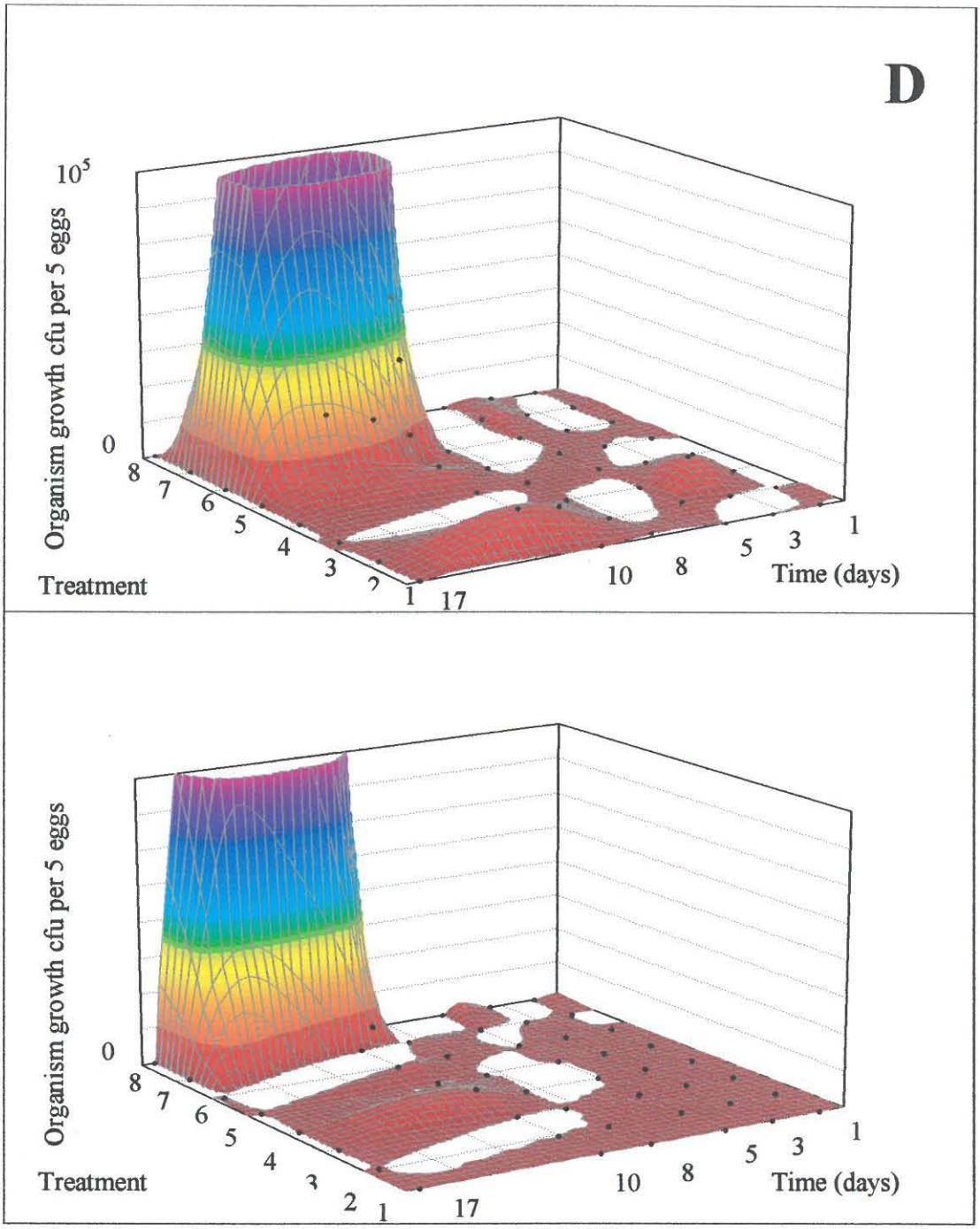


Fig. 4.4 Results of test for *Pseudomonas* spp in the egg contents (D) and on the eggshell (d) during treatments 1-8 for the duration of 17 days.

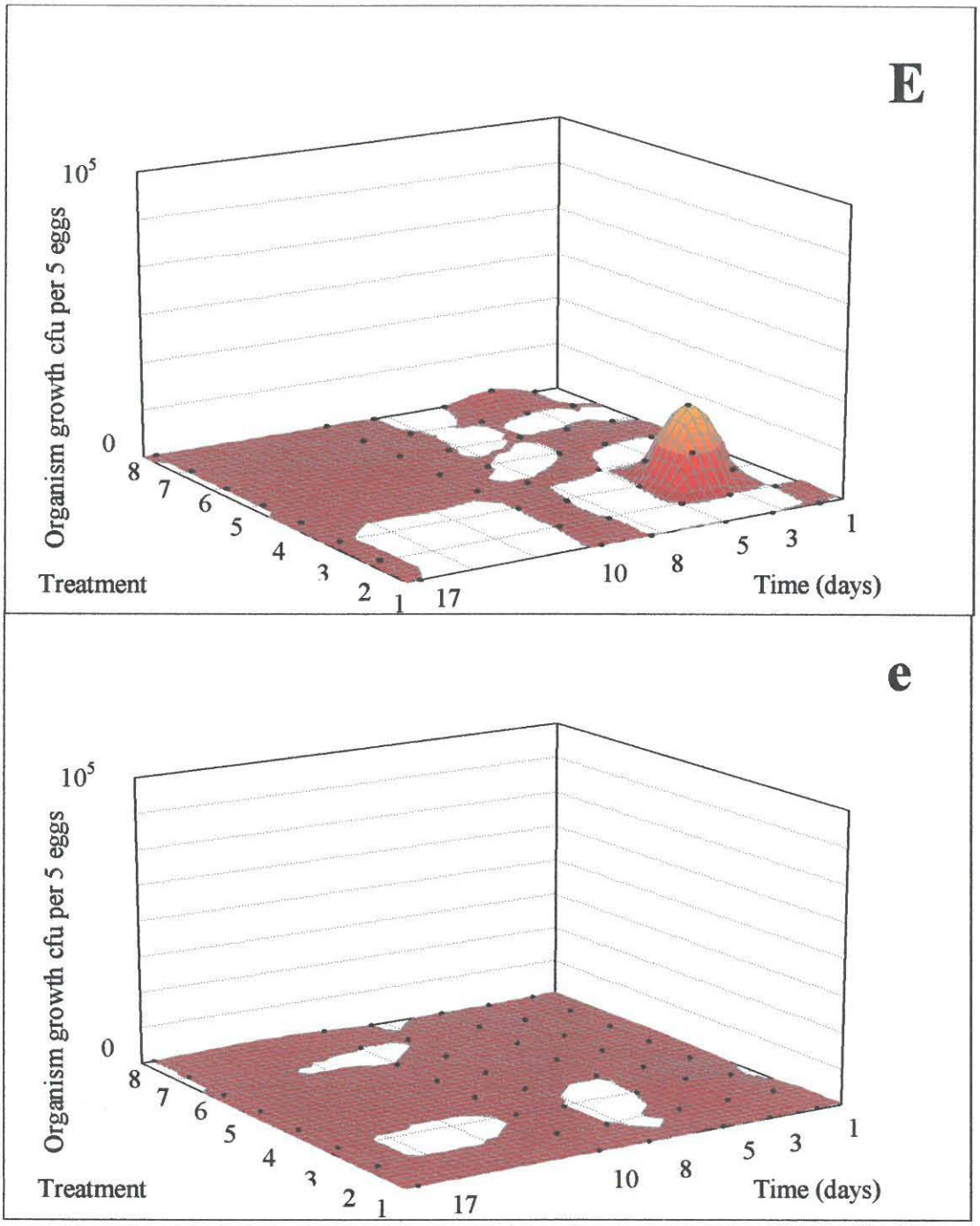


Fig. 4.5 Results of test for *Staphylococcusa* spp in the egg contents (E) and on the eggshell (e) during treatments 1-8 for the duration of 17 days.

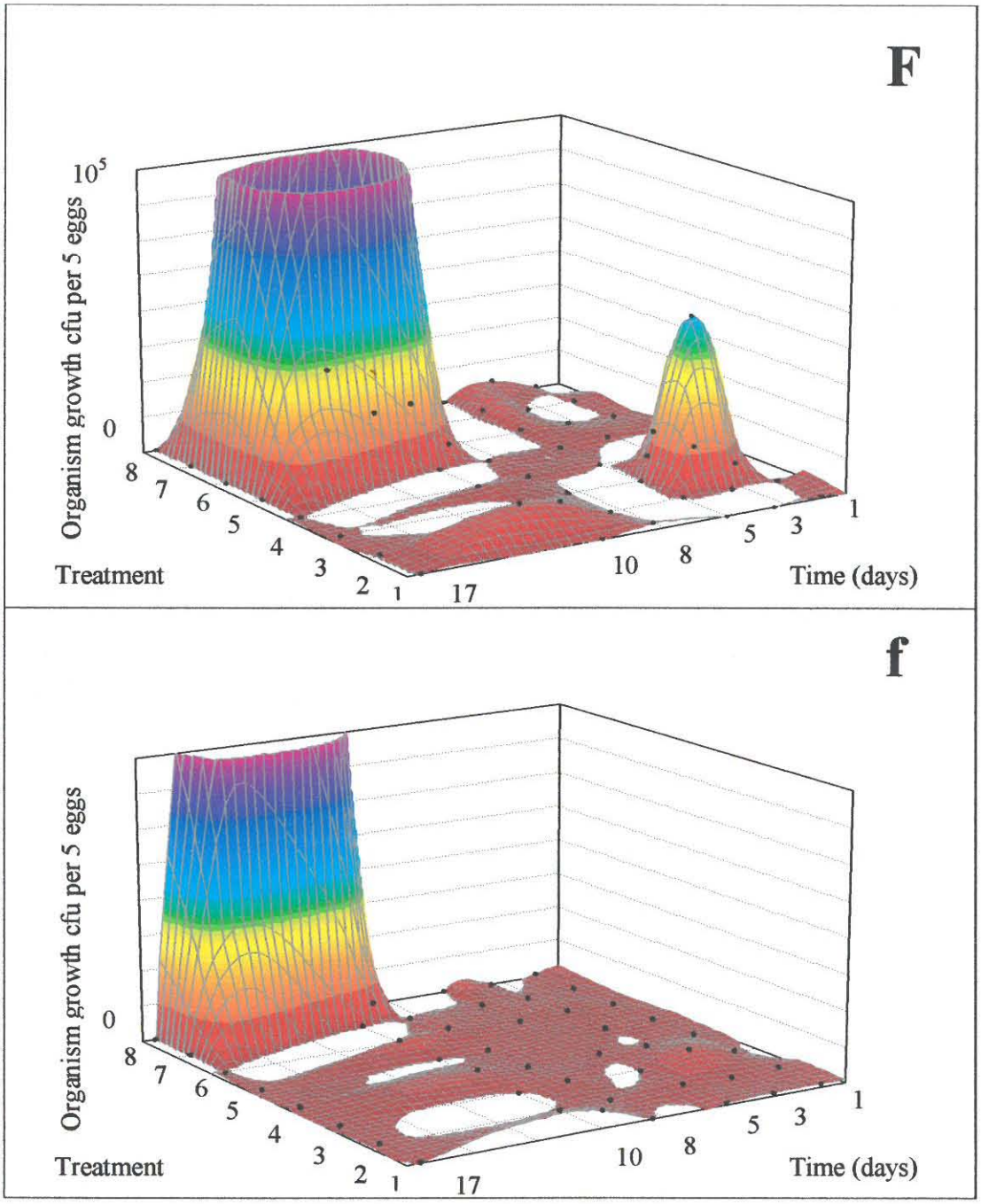


Fig. 4.6 Results of test for aerobic plate counts in the egg contents (F) and on the eggshell (f) during treatments 1-8 for the duration of 17 days.

Low temperature (4°C) shock (Treatments 3-5) (Figs 4.1A-4.6F)

No growth was noted in eggs that had been subjected to a 4°C shock for longer than 4 hours (treatments 4 and 5). The 2-hour, low temperature shock (treatment 3) resulted in a growth with a shortened lag phase (2 days) in the case of Total coliforms, *Pseudomonas* spp, *Staphylococcus* spp (Fig. 4.5E) and APC as shown in Figs 4.1A-4.6F. These organisms exhibited a typical *R* growth strategy showing a short lag phase (Atlas and Bartha, 1993). The "short" (compared to the control experiments) lag phase is followed by rapid exponential growth, with almost no stationary phase and then death. This change observed in the growth pattern was limited to *Salmonella* spp and was not noticeable for *E. coli* (Fig. 4.1A).

High temperature (37°C) shock (Treatments 6-8) (Figs 4.1A-4.6F)

The high temperature shock did not change the growth patterns of any of the analysed organisms compared to the control experiments. As illustrated in Fig. 4.4D, the maximum growth rate of *Pseudomonas* spp increased by one log phase to 2 x 10^5 cfu per 5 eggs after the 4-hour heat shock. This growth pattern was also observed for *E. coli* (Fig. 4.1A) and *Salmonella* spp (Fig. 4.2B) after similar treatment. Total Coliform counts showed that coliform organisms were reluctance to grow after the 4 and 6-hour heat shock. This treatment apparently inhibited the growth of *Staphylococcus* spp (Fig. 4.5E) completely, since no organisms of this kind were found. Results from the total aerobic plate counts (APC; Fig. 4.6F) seem to indicate that the growth pattern exhibited by the aerobic bacteria after heat shock treatment was very similar to that of *Pseudomonas* spp. Similar to treatments 1 and 2 for the egg contents, the heat shock resulted in no lag phase adjustment. The obvious higher

growth led to the presumption that the antimicrobial defence mechanisms in the egg was delayed. In all cases (treatments 1-8) the egg inhibited microbial growth, as little growth was observed on day 17.

4.4.2 Bacterial growth and interactions on the eggshell

High and *Low Temperature Control (Treatments 1* and *2)* (Figs 4.1a-4.6f)

Results showed that bacterial growth was limited by both control treatments (Fig. 4.1a-4.6f). Similar to what was observed with the egg contents, results of the total aerobic plate counts (APC) showed that after treatment 1 organisms on the eggshell exhibit a *K* strategic growth pattern (Atlas and Bartha, 1993), since marked growth occurred after a prolonged lag phase of 8 days. The growth patterns of *Pseudomonas* spp (1.000) and *E.coli* (1.000) showed high correlation with that of the aerobic bacteria. High temperature control at 25°C apparently affected the growth of *Staphylococcus* spp negatively, since no marked growth was observed for these organisms.

Low temperature (4°C) shock (Treatments 3-5) (Figs 4.1a-4.6f)

Limited bacterial growth was observed after eggs had been subjected to low temperature shocks. Similar to what was observed with the egg contents, organisms on the eggshell exhibited a *R* growth strategy (Atlas and Bartha, 1993) after the 2-hour low temperature shock, although this occurred in a more limited degree. The short lag phase of 2 days exhibited by coliform (Fig. 4.3c) and aerobic bacteria (Fig. 4.6f) on the eggshells was followed by exponential growth. The stationary phase was almost absent before death occurred. This observable change in the growth pattern was less pronounced for *Staphylococcus* spp (Fig. 4.5e) and not noticeable for

Salmonella spp (Fig. 4.2b) and *E. coli* (Fig. 4.1a). During treatment 3 the APC showed a high correlation with *Staphylococcus* spp (0.958), Total coliforms (0.951), *Salmonella* spp (0.951) on the eggshell. No growth was noted for *Pseudomonas* spp and *E. coli*.

In contrast to the egg contents, the eggshell exhibited a high temperature control growth pattern (*K* strategic) during the 4-hour 4°C shock. Limited exponential growth occurred on day 10 as shown in the case of *Pseudomonas* spp (Fig. 4.4d), Total coliforms (Fig. 4.3c) and the APC (Fig. 4.6f). With the exception of *Salmonella* spp (0.408) and *Staphylococcus* spp (0.159), APC correlated strongly with *Pseudomonas* spp (0.878), Total coliforms (0.876) and *E. coli* (0.878). No growth occurred on the eggshell with any of the microorganisms subjected to the 6-hour low temperature (4°C) shock.

High temperature (37°C) shock (Treatments 6-8) (Figs 4.1a-4.6f)

Compared to the high temperature control experiment for the eggshell, the 2-hour heat shock did not change the growth strategy of any of the organisms. *E. coli* counts (0.832) showed a high correlation with APC after the 2-hour heat shock. In the case of the 4-hour heat shock, bacterial growth patterns were different from what was found in the egg contents after the same treatment. Limited growth occurred with the exception of *Salmonella* spp (Fig. 4.2b) on day 5. This growth pattern resembled the growth of *Salmonella* spp (Fig. 4.2B) on day 10 in the egg contents. However, Total Coliform counts (0.164) showed low correlation with aerobic plate counts during the 4-hour heat shock.

After the 6-hour heat shock (treatment 8) growth of *Pseudomonas* spp growth increased by one log phase, to 2 x 10^5 cfu per 5 eggs (Fig. 4.4d) which resembled the growth in the egg contents, although growth increased from treatment 6 in the egg contents. A similar pattern was revealed by the Total coliform and the aerobic plate counts (APC) which showed that growht of these organisms also increased by one log phase. This trend was, however, not observed in the case of *Staphylococcus* spp, *Salmonella* spp or *E. coli*. Similar to treatment 1 and 2 for the eggshell, the heat shock resulted in no lag phase adjustment and the ability of the natural defence mechanisms of the eggs against microbial growth decreased. In all cases (treatments 1-8) little viability was noted on day 17 which implies that either the eggshell lost moisture and available nutrients, or that the eggshell gained control over microbial contaminants.

4.5 Discussion

In general higher counts of the organisms quantified in this study occurred in the egg contents than on the shell of the same simulations. In contrast to reports by Wang and Slavik (1988) that the rate of egg quality decline is lower if eggs are kept at temperatures close to freezing point, the results shown in this paper indicate higher *Pseudomonas* spp, Total coliforms and APC counts in eggs stored at 8°C than those eggs stored at 25°C. The results further demonstrate that treatments 4 and 5 (cold shocks) were most favourable for storage and transport purposes because organism growth was limited. During treatment 6-8 where eggs were subjected to high temperature shocks (temperature abuse) the growth rates were significantly higher.

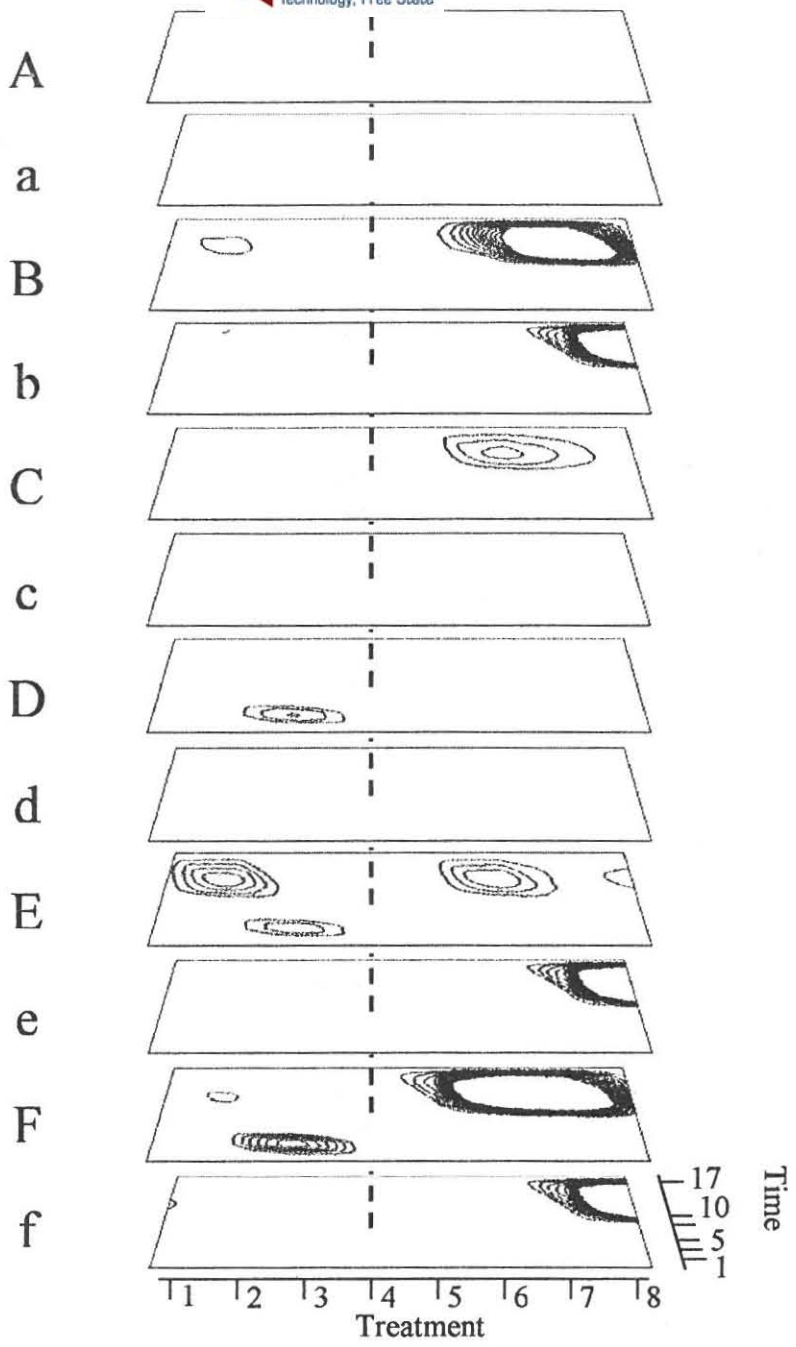


Fig. 4.7 A composite contour graph comparing the eggshell and egg contents of all the organisms investigated to actualise the treatment with the least growth during the study. A broken line shows the most favourable condition for storage and transport purposes. A: *E. coli* in egg contents, a: *E. coli* on eggshell; B and b: *Salmonella* spp, C and c: Total coliforms, D and d: *Pseudomonas* spp, E and e: *Staphylococcus* spp and F and f: Total aerobic plate counts (APC).

The microbial growth strategy observed with the brief cold shock period could prove beneficial in the control of microorganisms such as *Pseudomonas* spp and Total coliforms. Longer exposure to these low temperature conditions resulted in an almost complete termination of microorganism growth within the 17-day test period. Fig. 4.7 shows the combined effect of the various temperature shocks on growth on and inside chicken eggs. The exact reason for the growth strategy that changes due to brief exposure the cold shock is not yet clear.

Furthermore, Fig. 4.7 suggests the most favourable environment for storage and transport for eggs to be treatment 4 (broken line). During this treatment limited microbial growth occurred on the eggshell (which resulted in negligible growth) compared to the other treatments investigated during this study.

In this study it was shown that storage and transport conditions exerted considerable influence on the growth paterns of microbial populations on eggshells. Microbial egg quality improved in eggs that received a low temperature shock for 4 to 6 hours. However, a high temperature (37°C) shock simulating temperature abuse during storage and transport may lead to a considerable decrease in the microbial quality of eggs. Consequently under high temperature conditions an initial contamination of eggs with microorganisms such as *Pseudomonas* spp and *Salmonella* spp could be stimulated to multiply to numbers that will adversely affect shelf-life and product safety. According to South African legislation (Act 54 of 1972) Total coliform counts are not allowed to exceed 100 cfu.g^{-1} or 100 cfu.ml^{-1}, *Salmonella* counts 25 cfu.g^{-1} or 25 cfu.ml^{-1}, while no *Staphylococcus* organisms are permitted in egg and egg

products. It was noted in all instances that legislation was conformed in this study. It was further concluded that contrary to practices in the USA and European Community (Schoeni *et al.*, 1995) storage and transport at a constant temperature of 8°C proved not to ensure safer eggs in terms of storage and transport than at a constant 25°C. It was finally recommended to the egg industry that the incorporation of a cold shock of between 4 and 6 hours and consequent storage at 25°C would contribute to a longer egg shelf-life and enhanced egg quality.

4.6 References

Alaboudi, A.R., Hammed, D.A. and Ali, D.S. 1988. Microbial content of market eggs. *Indian Journal of Animal Sciences* 58, 768-770.

Atlas, R.M. and Bartha, R. 1993. Microbial communities and ecosystems. *In*: Atlas, R. M. and Bartha, R. eds. *Microbial Ecology: Fundamentals and Applications*, (3rd ed.) (pp.131-132). Benjamin/Cummings Publishing Company, Inc.: Redwood City, CA.

Chang, Y.H. 2000. Prevalence of *Salmonella* spp in poultry broilers and shell eggs in Korea. *Journal of Food Protection* 63, 655-658.

Ching-Lee, M.R., Katz, A.R., Sasaki, D.M. and Minette, H.P. 1991. *Salmonella* egg survey in Hawaii: evidence for routine bacterial surveillance. *American Journal of Public Health* 81, 764-766.

Frazier W.C. and Westhoff D.C. 1988. Contamination, preservation, and spoilage of eggs. *In*: Frazier, W.C. and Westhoff, D.C. eds. *Food Microbiology* (4th ed.) (pp.255-267). McGraw-Hill Book Company: New York.

Gentry, R.F. and Quarles, C.L. 1972. The Measurement of bacterial contamination on eggshells. *Poultry Science* 51, 930-933.

Goto, S. and Enomoto, S. 1970. Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of *Pseudomonas aeruginosa*. *Japanese Journal of Microbiology* 14, 65-72.

Hammack, T.S., Amaguana.R.M. and Andrews,W.H. 2001. Rappaport-Vassiladis medium for recovery of *Salmonella* sp. from low microbial load foods: collaborative study. *Journal of AOAC International* 84, 65-83.

Herbert, R.A. 1990. Methods for enumerating microorganisms and determining biomass in natural environments. *In*: Grigorova, R. and Norris, J.R eds. *Methods of Microbiology*. (pp. 1-39) Academic Press: New York.

Kamel, B., Bond, C. and Diab, M. 1980. Egg quality as affected by storage and handling methods. *Journal of Food Quality* 3, 261-273.

Lee, J. 2000. New *Salmonella* finding – inter-bacterial communication! *Agricultural Research* 48(1), 10-11.

Manafi, M. and Kneifel, W. 1989. A combined chromogenic-fluorogenic medium for the simultaneous detection of Total coliforms and *E. coli* in water. *Zentralblatt fur Hygiene* 189, 225-234.

Maré, L., Van Der Walt, M.L. and Dicks, L.M.T. 2000. Phage types of *Salmonella* enterica serovar *enteritidis* isolated in South Africa from 1991-1995. *Onderstepoort Journal of Veterinary Research* 67, 129-133.

Martley F.G., Jayashankar S.R and Lawrence R.C. 1970. An improved agar medium for the detection of proteolytic organisms in total bacterial counts. *Journal of Applied Bacteriology* 33, 363-370.

Nikanen, A. and Aalto, M. 1978. Comparison of selective media for coagulase-positive enterotoxigenic *Staphylococcus aureus*. *Applied and Environmental Microbiology* 35, 1233-1236.

Osborn, W.W. and Stokes, J.L. 1995. *Applied Microbiology* 3, 295.

Pienaar, A.C.E., Coetzee, L. and Bragg, R.R. 1995. A rapid method to determine bacterial contamination on hatching eggs. 2. Correlation of the optical-density measurements after incubation to bacterial counts on hatching eggs. *Onderstepoort Journal of Veterinary Research* 62, 25-33.

Radkowski, M. 2001. Occurrence of *Salmonella* sp. in consumption eggs in Poland. *International Journal of Food Microbiology* 64, 189-191.

Schultze, G.E. and Fawcette, H.A. 1996. Prevalence of *Salmonella enteritidis* in poultry shell eggs in Arkansas[*]. *Southern Medical Journal* 89, 889-891.

Schoeni, J.L., Glass, K.A., McDermott, J.L. and Wong, A.C.L. 1995. Growth and penetration of *Salmonella enteritidis*, *Salmonella heidelberg and Salmonella typhimurium* in eggs. *International Journal of Food Microbiology* 24, 385-396.

South Africa. 1972. *Foodstuffs, Cosmetics and Disinfectants Act, 1972 No. R.692 of 16 May 1997*. Government Printers: Pretoria.

Todd, E.C.D. 1996. Risk assessment of use of cracked eggs in Canada. *International Journal of Food Microbiology* 30, 125-143.

Trepka, M.J., Archer, J.R., Altekruse, S.F., Proctor, M.E. and Davies, J.P. 1999. An increase in sporadic and outbreak-associated *Salmonella Enteritidis* infections in Wisconsin: The role of eggs. *The Journal of Infectious Diseases* 180, 1214-1219.

Vassiliadis, P. 1983. The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of *Salmonellas*: An overview. *Journal of Applied Bacteriology* 54, 69-76.

Venter, P., Lues, P.F.R. and Theron, H. 2003. The quantification of bioaerosols in automated chicken egg production plants. *Food Research International* (submitted for publication).

Wang, H. and Slavik, M.F. 1998. Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. *Journal of Food Protection* 61, 276-279.

Zeidler, G. 1995. What makes microorganisms grow on eggs and in the egg room? (Part I). *Poultry Bulletin* 12, 363-365.

Chapter 5

GENERAL CONCLUSIONS

5.1 Introduction

With limited information available on the bacterial quality of chicken eggs produced in South Africa the purpose of this study was to quantify and identify potential microbial hazards associated with chicken eggs produced in the Central Free State region. Chapter 2 reported on the investigation of the microbial quality of the shells and contents of eggs from a controlled as well as an uncontrolled layer management system (LMS) were investigated in order to assess the influence of facility design on the level of microbial contamination and growth rate. In the second phase of the study, reported in Chapter 3, the levels and distribution of viable airborne microorganisms, as well as the effect of facility design on the bioaerosol composition were investigated. This was done to determine the sources of microbial contamination identified in Chapter 2. Chapter 4 reported on the final phase of the study, when the eggs were exposed to various transport and storage simulations. The objective was to determine the influence of temperature abusive conditions on the bacterial growth and thus egg quality and safety.

5.2. Concluding remarks on Chapters 2, 3 and 4

In Chapter 2 the microbiological hazards associated with eggs from two different layer management system (LMS) designs were compared. Eggs from both systems contained relatively low levels of spoilage organisms compared to the results of Frazier and Westhoff (1988) where the total number of viable microorganisms ranged from 10^2 to 10^7 per eggshell. Based on the *Salmonella* spp, coliforms and *E. coli* counts, the contents of the eggs were generally more contaminated in the uncontrolled LMS. Unrestricted airflow in the uncontrolled LMS probably caused more fungal spores to be present on the eggs. The controlled LMS, with its automated fecal matter disposal system, showed higher levels of fecal-related contaminants on the eggshells compared to the uncontrolled LMS with its open-air lower level. It appears that facility design influenced the microbiological quality as well as microbial growth rate on the eggs, since both systems housed hens from the same breed namely Highline Silvers which came from the same distributor and were ssubjected to the same feeding protocol.

The microbial composition of the bioaerosol in the two layer management systems were subsequently compared, using an air sampling procedure, as it was important to characterise the microbiota predominating in the production environment (Lutgring *et al.*, 1997). In terms of the fecal control system and ventilation, facility design was found to have influenced the level of culturable airborne bacteria, which could further have influenced egg quality and safety should the airborne bacteria have settled on the eggs. Although an automated fecal matter disposal system decreased labour requirements at the controlled LMS, this study revealed that aerosolised fecal matter

scraped from the conveyer belts was a prominent source of bioaerosols contamination. It is therefore important to remove all eggs from the layer house before the fecal matter is scraped from the conveyer belts.

Various storage and transport conditions simulated in this study showed that temperature influenced the microbial proliferation patterns on the chicken eggs. In general, microbial egg quality improved when eggs received a low temperature shock for 4 to 6 hours. In contrast, a high temperature (37°C) shock during storage and transport, lead to a dramatic decrease in the microbiological quality of the eggs. This implies that an initial contamination of eggs with microorganisms such as *Pseudomonas* spp and *Salmonella* spp could be stimulated to proliferate to numbers that will affect shelf-life and safety of the product. This study has proven that storage and transport at a constant temperature of 8°C a practice employed by both the European Community and the United States according to Schoeni *et al.* (1995) does not necessarily yield safer eggs than storage and transport at a constant temperature of 25°C.

5.3. Recommendations to industry

Based on the conclusions a simple and applicable solution to limit the contamination of the eggs from the controlled LMS was suggested, namely that eggs should be extracted from the automated egg removal mechanism prior to the scraping and removal of the faeces. This procedure would limit the aerosolization of fecal particles and the associated microorganisms which could settle on the eggs prior to collection. When this fecal aerolisation is limited in the controlled system, it is likely to produce eggs of higher microbiological quality.

The results further indicated that chickens of both systems probably carried bacteria, that could have contributed to the contamination of the eggs. It is therefore necessary to screen the chicken feed in particular, as well as other possible sources of microbiota for the presence of foodborne pathogens. Such results will indicate corrective actions required to ensure healthier chickens.

The egg industry could further increase egg quality by incorporating a cold shock (4°C) treatment of between 4 and 6 hours followed by storage of the eggs at 25°C. According to results obtained in this study this treatment limits microbial growth and contributes to an increased shelf-life.

5.4 Future research

The following were identified as possible future research projects:

- microbiological hazard identification of chicken eggs from a wider spectrum of commercial settings in South Africa;
- microbiological hazard identification of chicken eggs associated with backyard and free-range chickens from specific areas in the Free State and other parts of South Africa and how this with layer management systems;
- studies into the viable airborne microorganisms (bioaerosols) in different commercial egg producer plants, especially the packaging facilities of various egg producer plants and comparing the results;
- determining the direct effect of airborne contamination on egg shelf-life and quality; and
- evaluation of the handling and cooking methods of eggs from backyard and free-range chickens using means of questionnaires.

5.5 References

Frazier W.C. and Westhoff D.C. 1988. Contamination, preservation, and spoilage of eggs. *In*: Frazier, W.C. and Westhoff, D.C. eds. *Food Microbiology* (4th ed.) (pp.255-267). McGraw-Hill Book Company: New York.

Lutgring, K.R., Linton, R.H, Zimmerman, N.J. Peugh, M and Heber, A.J. 1997. Distribution and quantification of bioaerosols in poultry-slaughtering plants. *Journal of Food Protection* 60, (7) 804-810.

Schoeni, J.L., Glass, K.A., McDermott, J.L. and Wong, A.C.L. 1995. Growth and penetration of *Salmonella enteritidis, Salmonella heidelberg and Salmonella typhimurium* in eggs. *International Journal of Food Microbiology* 24, 385-396.