Some preslaughter feeding and other environmental effects on aspects of gut microbiology of cattle and chickens.

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Abstract

Controlling microorganisms, especially pathogenic bacteria, in meat-producing animals destined for slaughter is important for reasons of consumer food safety, profitability and animal welfare. It is difficult for the consumer to accurately assess the safety of meat products, which means that meat must be provided with some form of assurance that it will be safe to eat. The overall objective of this work was to investigate approaches to improving food safety through preslaughter manipulation of gut microorganisms in cattle and chickens. The preslaughter feeding treatment of cattle, which offered the most advantages in the first study, was the provision of hay for 48 hours before despatch to slaughter. This method helped to reduce the gut burden and excretion of *Escherichia coli (E. coli)* and it helped keep the animals clean. Cattle that were transported directly from pasture had runny faeces and ended up with more surface soiling on the hide. Fasted animals produced less effluent during transport, but they had high levels of *E. coli* in their rumens and faeces at slaughter. The way the cattle were fed before slaughter had little effect on the amount of weight they lost. These results were confirmed in the second study involving eight preslaughter feeding regimes, with cattle fed red clover hay for 48 hours prior to transport to slaughter having reduced *E. coli* counts in the rumen to less than 1 log count g^{-1}. It is recommended that preslaughter fasting of cattle be reduced to 18 hours or less, including transport, to minimize gastrointestinal *E. coli* counts at slaughter and to minimize losses in carcass weight. The addition of commercial additives (a pre- and a syn-biotic) to the diet of chicks in their growing environment improved the chick growth rates and weights, however it also caused increased *Eimeria tenella* infection, following a challenge, resulting in significantly higher lesion scores. The presence of hens imparted partial resistance to infection to the chicks, but negatively affected their growth rates compared to chicks raised without hens.

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Chapter 1

Introduction

The importance of controlling microorganisms, especially pathogenic bacteria, in meat-producing animals destined for slaughter must be appreciated for reasons of consumer food safety, profitability and animal welfare. The extent to which a meat quality characteristic influences a consumer depends on the accuracy with which it can be assessed by the consumer and the degree to which it is likely to deviate from a satisfactory level (Purchas *et al.*, 1989). Meat quality characteristics, such as safety and wholesomeness characteristics, are of high importance to consumers. A safe product is one which will not adversely affect the health of the consumer and a wholesome product is one which complies with the characteristics the public expects, even if the presence of an undesirable defect does not create a health hazard. However, it is very hard for the consumer to accurately assess the safety of meat products. This means that meat that is produced must be provided with some form of assurance that it will be safe to eat. Relevant aspects of, and interrelationships between, food safety, profitability and animal welfare are outlined in this introduction.

1.1 FOOD SAFETY

Food-borne illnesses are among the most widespread diseases of the contemporary world. It is estimated that seven million people a year are affected by food borne illness and, that 7000 die (Byrne, 1998). Many agents of food-borne disease are carried by red meat animals and poultry but there are wide differences between and within species in the incidence of affected animals and numbers of bacteria present (Mackey, 1989). Preventing humans from suffering the effects of food contamination clearly starts at the agricultural stage, and this is not only true of livestock, but also crops (Byrne, 1998). The chain of events involved in primary production, harvesting, processing, distribution and final preparation is quite long, and there are many opportunities for the food to become contaminated (Notermans, 1999). It is important to attempt to minimise the carriage of pathogens in live animals and the contamination of carcasses in the processing plant.

Markets demand safe, wholesome products because food safety is top priority among consumers. Food manufacturers need to be assured that the raw materials they use are wholesome (Byrne, 1998). Contamination of beef and poultry products leading to food poisoning in consumers could cause decreased consumption of these products. Incidents such as the *Escherichia coli* O157:H7 food poisoning outbreak in Scotland that killed twenty people and outbreaks of food poisoning caused by *Salmonella* and other bacteria, have threatened food supplies in Japan, the United States of America and the United Kingdom, and have shaken consumer confidence in the agro-food industry (Byrne, 1998).

The beef and poultry meat industries are very important economically, to New Zealand, both domestically and internationally. In 1999-2000, New Zealand exports of beef and veal made up 8% of the world export market, worth $1400.4 million with major beef markets including the United States, Canada, Japan, South Korea and Taiwan (Anon, 2001). In 1998, the New Zealand poultry industry produced 100,000 tonnes of poultry meat, almost solely for the domestic market, earned almost $500 million in retail sales, and provided about 3000 jobs (PIANZ, September 2001).

Decreasing the number of foodborne pathogens carried in and/or on animals to the processing plant and therefore the chance of contamination will help to ensure that both the domestic and international markets for beef and poultry products continue to grow. Important pathogens in the microbial contamination of meat include *Salmonella* and *Escherichia coli*.

Salmonella causes hundreds of thousands of foodborne infections each year by contaminating meat and other foodstuffs of animal origin (Oosterom, 1991; Portillo 2000). The number of *Salmonella* infections has steadily increased in the last few decades, mainly due to the continuous growth of industrialisation in animal husbandry, slaughter procedures and food processing (Oosterom, 1991). *Salmonella* infections lead to a variety of diseases known as salmonellosis.

Escherichia coli may be the most versatile of human pathogens (Donnenberg and Nataro, 2000). *E. coli* is a common organism found in the gastrointestinal tract of warm

blooded animals and humans (Mermelstein, 1993). This organism is not only the dominant gram-negative facultative anaerobe in the human gastrointestinal tract, it is also a potent pathogen capable of a variety of diseases by an array of mechanisms (Donnenberg and Nataro, 2000). Some strains can produce enteric, urinary tract and wound infections as well as food poisoning, and occasionally septicaemia and meningitis (Eley, 1996).

1.2 PROFITABILITY

Abattoirs do not like diseased or heavily contaminated animals. When meat has to be discarded because it is spoiled through contamination or disease, profitability is decreased. Microbial contamination of animal carcasses is a result of the necessary procedures required to process live animals into retail meat (Dickson and Anderson, 1992).

Making sure that there is a continuous supply of suitable stock to the killing floor has implications for line efficiency and animal welfare in four ways. Animal handling becomes more critical as the animals must arrive in a continuous stream at the stunning point (Gregory, 1998). Uncontrolled behaviour in the stock can create interruptions in this flow.

The animals must be healthy and free from blemishes. Diseased and bruised or blemished tissue needs to be removed, and the additional inspection and trimming this involves can slow the line or require extra trimming (Gregory, 1998). Extra work is involved in trimming the diseased tissues and if there are a lot of diseased animals the line speed will be reduced (Gregory, 1998). Under commercial beef slaughtering conditions, trimming may be a highly variable process, with its efficacy primarily related to the skill and/or diligence of the individual doing the trimming (Prasai *et* al., 1995; Reagan *et al.*, 1996). The physical contact with the carcass during trimming may contribute to additional contamination if the equipment has not been properly sanitised. Holding of the carcass for trimming at the warm slaughter room before final washing and chilling may allow for better attachment of bacteria (Reagan *et al.*, 1996). Trimming may facilitate bacterial penetration into carcasses.

Animals must be fasted to reduce gut contents to lower the risk of rupturing the digestive tract during evisceration, which would cause contamination of carcass with digesta and faeces (Gregory, 1998). Animals must be presented in a clean condition as stock which are dirty with dung, mud or dust on their surface create the risk of spreading dirt (Gregory, 1998). If dirty carcasses enter the dressing area, the veterinarian or supervising meat hygiene officer may be obliged to stop or slow the line in order to ensure either that the dirty carcasses are handled appropriately and do not contaminate equipment or other carcasses, or that further dirty stock do not enter the killing floor (Gregory, 1998).

1.3 ANIMAL WELFARE

Control of diseases in animals destined for human consumption is important for animal welfare in addition to its importance for food safety and profitability. Meat consumers have shown an increasing level of concern about the welfare implications of animal production systems over recent years. For ethical reasons alone, production animals should have as high a quality of life as possible, and certainly any treatment that may cause suffering is unacceptable. Preslaughter handling of animals and birds has a profound effect on the quality, and therefore, value of the meat (Varnam and Sutherland, 1995). Between farm and slaughter, cattle are subjected to transportation, confinement, unfamiliar surroundings and additional handling. Collectively or separately, these and other factors can constitute preslaughter stress.

Stress is an imprecise term, but it can be defined as an animal's response to any demand made upon it (Shorthose and Wythes, 1982). This definition is all-inclusive and implies that every demand made upon an animal causes a stress-related response. This is not necessarily true and therefore stress needs to be further defined. It can be described as an animal's response to conditions or factors that challenge its normal state of being. In animal husbandry, stress has usually been explained as a reflex action that occurs when animals are exposed to adverse conditions, and which is the cause of many unfavourable consequences, ranging from discomfort to death (Dantzer and Mormede, 1983). Moberg (1996) described stress as simply the biological responses that an animal uses to defend its homeostasis or biological status quo, from both external and internal challenges (or stressors). However, stress jeopardizes the animal's welfare only if it results in some

significant biological cost to the animal that places that individual's well-being at risk (Moberg, 1996). Dantzer and Mormede (1983) suggested that stress occurs when an animal has been exposed to adverse conditions. This could be interpreted as only external or environmental conditions such as, weather extremes. The use of "internal and external challenges" in Moberg's (1996) definition makes clear the possibility of stress being linked to environmental and internal stressors. Moberg (1996) also stressed that animal welfare is only jeopardized if stress results in significant biological cost to the animal.

Stress in the preslaughter period leads to increased defaecation and contamination of the bodies of animals and birds, resulting ultimately in an increased risk of contamination of the meat (Varnam and Sutherland, 1995). The incidence of *Salmonella* in pigs and poultry can increase considerably under these conditions. It has also been suggested that stress can lead to shedding of *Escherichia coli* O157:H7 by cattle which had previously tested as non carriers (Varnam and Sutherland, 1995).

One of the consequences of poor welfare associated with disease is that resistance to other disease is reduced (Fraser and Broom, 1990). Disease is one of the most important causes of animal suffering (Gregory, 1998). Animals that are diseased very often have difficulty coping with their environment, or fail to do so; hence their welfare is poorer than that of a healthy animal in otherwise comparable conditions (Fraser and Broom, 1990). Whether the disease causes pain or other kinds of discomfort or distress, treatment, which reduces the effects of the disease, is clearly improving the welfare or the animal (Fraser and Broom, 1990).

Coccidiosis is a protozoan disease of fowl and is a problem in all poultry-producing areas that has serious animal welfare implications. Despite recent advances in control and treatment, the disease remains one of the principal causes of economic loss to the poultry industry.

An understanding of the behaviour of animals will facilitate handling, reduce stress, and improve both handler safety and animal welfare (Grandin, 1989). 'Behaviour' is a term used widely in many sciences. Kilgour and Dalton (1984) describe behaviour as the patterns of action observed in animals that occur either voluntarily or involuntarily. An

animal's behaviour provides information on a wide range of factors such as breathing, eating, drinking, fighting, mating and milking (Kilgour and Dalton, 1984). Fraser and Broom (1990) suggest that farm animal behaviour research is relevant and necessary for animal production enterprises to be carried out effectively and economically. Observations of external behaviour can often lead to deductions about the internal state of the animal (Kilgour and Dalton, 1984).

1.4 OBJECTIVES

The overall objective of this work was to investigate approaches to improving food safety through preslaughter manipulation of gut microorganisms in cattle and chickens. Specific objectives were:

- To assess the effect of preslaughter feeding system on gastro-intestinal and faecal *Salmonella* and *E. coli* in steers and heifers.
- To measure the effect of preslaughter diet on faecal consistency and dry matter of the gastro-intestinal contents and faeces of steers.
- To determine the effect of preslaughter diet on the weight of contents in the gastro-intestinal tract of steers.
- To evaluate the effect of preslaughter feeding systems on weight loss in steers and heifers.
- To assess the effect of rearing chicks in the presence of adults on their ability to withstand *Eimeria tenella* infection.
- To determine the effect of growing environment on the weight of chicks before and after infection with *Eimeria tenella*.
- To evaluate the effect of rearing chicks in the presence of adults on their responses to a fearful situation.

Chapter 2

Literature Review

2.1 INTRODUCTION

The process of converting live meat animals into carcasses for further processing has been modernised, streamlined, quickened, and made more efficient (Siragusa, 1995). Most of the sectors of the food industry face a major and continuing challenge in trying to limit the extent to which food products become contaminated with pathogenic bacteria during primary processing (Sheridan, 1998).

Entry into the slaughterhouse is a critical control point with respect to food safety, and preventing infected animals entering the plant enhances the control of pathogens. Traditional methods of decontamination of animals and carcasses involve trimming and washing.

The manipulation of gastrointestinal flora of animals preslaughter or in the growing environment could lead to a reduction in meat borne pathogens entering the abattoir. This in turn could lead to improved food safety and decreased carcass contamination. Following is a review of *Salmonella* spp and *Escherichia coli*, important meat borne pathogens to control in terms of food safety and methods of carcass contamination and decontamination. Factors influencing gut flora and manipulation of gut flora in the preslaughter or growing period will also be discussed.

2.2 MEAT-BORNE PATHOGENS

Pathogens, like other microorganisms, are ubiquitous throughout nature (Berne, 1998). Several human-pathogenic bacterial species have their main reservoir in the animal kingdom. Two of the most important, in terms of human illness caused by consumption of contaminated meat products, are *Salmonella* and *E. coli*.

2.2.1 *Salmonella* species

Salmonella species are Gram-negative, facultatively anaerobic, non-spore-forming bacilli that can be split into more that 200 serotypes (Eley, 1996). It is generally accepted that virtually all *Salmonella* serotypes, if present in foods in sufficiently high numbers, may cause febrile gastroenteritis when absorbed with food (Mossel, 1977). The number of cells required for triggering food-borne illness depends on the *Salmonella* serotype in question, and the condition and age of the person consuming the food (Mossel, 1977). Diseases caused by salmonellae are divided into two major groups: (1) a localised, self-limiting bacterial infection of the intestinal epithelium, known as gastroenteritis; (2) a systematic infection known as 'enteric fever' (Portillo, 2000). The incubation period for salmonellae ranges from 5 hrs to 5 days, but signs and symptoms usually begin 12-36 hrs after ingestion of a contaminated food (ICMSF, 1996; Eley, 1996). The shorter incubation periods are usually associated with higher doses of the pathogen or highly susceptible persons. Signs and symptoms include diarrhoea, nausea, abdominal pain, mild fever and chills (ICMSF, 1996). The syndrome usually lasts 2-5 days and the excreta of infected persons will contain large numbers of salmonellae at the onset of illness (ICMSF, 1996).

Salmonella organisms are widely distributed in nature and are commonly found in the intestinal tracts of animals and human carriers (Eley, 1996). Environmental spreading of *Salmonella* is mostly brought about by free-living animals, by materials originating from slaughter animals, such as manure, slurry and intestines, and materials from the human population, such as sewage sludge and household refuse (Oosterom, 1991).

The handling of animals and animal products known to be contaminated or infected with salmonellae must be regarded as an occupational hazard (Hobbs, 1974). *Salmonella* contamination of meat is caused by the spreading of bacteria from the gut to the surface of the meat (Oosterom, 1991). The incidence of salmonella on carcasses is influenced by numerous factors such as carriage in the live animal, which in turn depends on husbandry practice; stress during transport; time spent in lairage and slaughter hygiene (Mackey, 1989).

A number of foodstuffs have been associated with salmonellosis, typically meat products (especially poultry), eggs, untreated milk and diary products, and any foods that have undergone faecal contamination (Oosterom, 1991; Eley, 1996). Salmonellae from raw products contaminate the area of food preparation and may be transferred to the cooked food by means of fingers, surfaces, utensils, equipment and other environmental factors (Hobbs, 1974). Cooked meat is not often a direct cause of infection, since in most cases superficial heating is enough to eliminate all *Salmonella* contamination present on the surface (Oosterom, 1991). This does not apply to meat that is minced or otherwise treated in such a way that contamination can reach the inside and escape the effects of heating (Oosterom, 1991).

The control of salmonellae in the food chain is a complex matter, because of the interrelationship between environmental contamination, farm animals and humans (Eley, 1996). For the sake of prevention it is essential to realise that all raw foods of animal origin and in addition, a few vegetables might be contaminated by salmonellae (Mossel, 1977). There are a number of ways of controlling the entry and spread of salmonellae in animals. These include regulated importation of live and slaughtered animals, use of salmonellae-free breeding stock and animal feeds, and good husbandry practices with egg laying and broiler flocks (Eley, 1996).

The education of consumers and of food-handlers on the safe handling and cooking of potentially hazardous meats and other raw ingredients is of paramount importance (Eley, 1996). Thoroughly heated foods should be protected from salmonella recontamination (1) by equipment that has been soiled by fresh meats or poultry, and (2) by growth of an occasional recontaminant, which can be prevented by adequate refrigeration (Mossel, 1977). Various procedures have been developed to destroy salmonellae in foods, including the use of heat, irradiation, acidification and combinations of factors (ICMSF, 1996).

2.2.2 *Escherichia coli*

E. coli is a common faecal contaminant, and there are no specific control measures for this organism. Evidence suggests that *E. coli* can multiply in food and that large numbers of organisms (e.g. 10^5-10^7 orgs/g) need to be present to cause infection (Eley,

1996). Smaller numbers of certain serotypes, such as O157:H7, only require very low numbers of organisms present to cause infection. After an incubation period of 12-72 hours, symptoms consist mainly of diarrhoea, sometimes with the presence of blood and/or mucus. Symptoms may vary in type and severity depending on the serotype of the organism (Eley, 1996).

The source of the organisms can be human as well as animal. The mode of foods becoming hazardous seems to be similar to that for salmonellosis (Mossel, 1977). Faecal contamination of foods, either by direct contact or indirectly via component water, is probably the most important method of transmission (Eley, 1996). This type of contamination is most likely to affect meats, meat products and fresh vegetables, which then become the source of infection (Eley, 1996). Food may be contaminated by infected food handlers who practise poor personal hygiene or by contact with water contaminated by human sewage (ICMSF, 1996).

Special emphasis needs to be placed on avoiding direct faecal contamination of food by stressing strict personal hygienic practices, especially by food handlers (Eley, 1996). Important measures to prevent food poisoning include properly heating foods to kill pathogens and holding foods under appropriate conditions to avoid bacterial multiplication (e.g. chilling of leftover foods rapidly) (ICMSF, 1996). Meat dishes such as minced meat, rolled roasts and whole poultry must be cooked thoroughly to a temperature of at least 75°C (Anon, 1997). It is safe to eat meat 'rare' when it is in the form of whole, unrolled cuts of meat like steak, because the *E. coli* bacteria only live on the meat surface and are killed by cooking temperatures (Anon, 1997).

E. coli have been subdivided into a number of groups. The four most important groups are: (1) Enteropathogenic (EPEC), (2) Enteroinvasive (EIEC), (3) Enterotoxigenic (ETEC), and (4) Enterohaemorrhagic (EHEC).

2.2.2.1 Enteropathogenic E. coli (EPEC)

Enteropathogenic *E. coli* (EPEC) cause characteristic pathological lesions in the intestine similar to those produced by *E. coli* O157:H7 (a member of the EHEC group), but can be differentiated from those produced by other types of *E. coli* (ICMSF, 1996).

Enteropathogenic strains of *E. coli* cause a bloody diarrhoea which, in some cases, may be prolonged (Madden, 1994). This type of gastroenteritis generally attacks infants and is produced by strains of the microbes that produce the shiga-like toxin (Madden, 1994).

2.2.2.2 Enteroinvasive E. coli (EIEC)

Enteroinvasive *E. coli* (EIEC) closely resemble *Shigella* in pathology, biochemical activity and antigenicity (ICMSF, 1996). The organisms differ from most *E. coli* in that they ferment lactose slowly, or not at all, may be anaerogenic and are non-motile. They attack the colonic mucosa, invading epithelial cells, multiplying and eventually causing ulceration of the bowel (ICMSF, 1996). Illness is characterised as a mild form of dysentery, often mistaken for shigellosis (Madden, 1994). Blood and mucus in the stool of infected individuals is characteristic of this form of dysentery, which is generally self-limiting (Madden, 1994).

2.2.2.3 Enterotoxigenic E. coli (ETEC)

Enterotoxigenic *E. coli* (ETEC) are a major cause of infantile diarrhoea in developing countries and are the leading cause of traveller's diarrhoea in many developed countries (ICMSF, 1996). In contrast to EPEC, which colonise the entire intestine, ETEC are limited to the proximal small intestine (ICMSF, 1996). This *E. coli* strain causes watery diarrhoea, abdominal cramps, low-grade fever, nausea and malaise (Madden, 1994). This type of diarrhoea is generally self-limiting.

2.2.2.4 Enterohaemorrhagic E. coli (EHEC)

Escherichia coli O157:H7 was first identified as a cause of illness in 1982 during an outbreak of severe bloody diarrhoea that was traced to contaminated hamburgers (Tuttle *et al.*, 1999). Three different diseases have been attributed to *E. coli* O157:H7: haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombocytopenic purpura (TTP) (Reid, 1991). Haemorrhagic colitis is characterised be severe crampy abdominal pain followed within 24hrs by watery diarrhoea, which later becomes grossly bloody (Reid, 1991; Eley, 1996). Abdominal pain may be so intense as to resemble appendicitis. Patients then develop serious blood disorders that lead to renal failure.

This disease is a leading cause of acute renal failure in children (Reid, 1991). Patients often require blood transfusions and dialysis, and may develop central nervous system disorders resulting in seizures, coma and possibly death (Reid, 1991). Vomiting may occur but there is little or no fever, with the symptoms lasting from 2 to 9 days (Reid, 1991; Eley, 1996).

Ten percent of cases develop into a serious illness called haemolytic uraemic syndrome (HUS), which consists of a triad of symptoms: acute renal failure, thrombocytopaenia and microangiopathic haemolytic anaemia (Eley, 1996). Patients often require dialysis and may suffer permanent kidney damage. The symptoms of thrombotic thrombocytopenic purpura are similar to those of HUS in that the central nervous system is affected (Reid, 1991). Some patients develop blood clots in the brain, which are often fatal (Doyle, 1991).

In the UK the first report of isolation of *E. coli* O157:H7 from bovine faeces was in 1989 (Eley, 1996). Many strains of *E. coli* are known to cause human disease but animals are not believed to be the source of infection (Mackey, 1989). In contrast, epidemiological evidence strongly suggests that cattle may act as a reservoir for *E. coli* O157:H7 and the organism has been recovered from healthy cows (Mackey, 1989). *E. coli* O157:H7 is generally associated with dairy cattle and their products, milk and beef, but it has also caused outbreaks associated with water and apple cider (Berne, 1998; Mermelstein, 1999).

Prevention of faecal contamination during the recovery and processing of food derived from animals is paramount in controlling food-borne *E. coli* O157:H7 infections (ICMSF, 1996). Reducing the risk of contamination hinges on limiting carcass contamination during slaughter and dressing (Anon, 1997). Control of chilling and transport temperatures is essential to prevent the growth of any contaminating enterohaemorrhagic *E. coli* (Anon, 1997). As the dose required for infection with *E. coli* O157:H7 is very low it is necessary to enforce a zero tolerance of this organism in processed food (Tuttle *et al.*, 1999).

The control of food-borne diseases depends on understanding their mechanism of transmission well enough to prevent it (Notermans, 1999). A knowledge of factors

contributing to food-borne disease facilitates the identification of specific control measures, and hence the prevention of such disease (Notermans, 1999). It is important that we attempt to control the carriage of potential food poisoning organisms in the live animal and minimise the opportunities for contamination of carcasses in the abattoir or processing plant (Mackey, 1989). Knowledge of the characteristics of food-borne organisms enables the identification of suitable conditions for food processing and preparation and if these conditions are well-selected, the growth of microorganisms can be inhibited or the organisms can be destroyed (Notermans, 1999).

2.3 CARCASS CONTAMINATION

Microbial contamination of animal carcasses is a result of the necessary procedures required to process live animals into retail meat (Dickson and Anderson, 1992). The process of converting live meat animals into carcasses for further processing has been modernised, streamlined, quickened, and made more efficient (Siragusa, 1995). Most of the sectors of the food industry face a major and continuing challenge in trying to limit the extent to which food products become contaminated with pathogenic bacteria during primary processing (Sheridan, 1998). Control of pathogens needs to be addressed at all stages of production, processing and preparation. It has been long recognised that the slaughter stock themselves are a major source of carcass contamination (Empey and Scott, 1939).

The living animal can be considered to be exposed to microorganisms from inside (i.e. the gastro-intestinal tract with a natural and sometimes unnatural burden of microorganisms) and the outside (the environment in which it lives). In the normal healthy animal most of the tissues, which ultimately become meat or meat products, are sterile, however fresh meat presents an environment which is ideal for the growth of many microbes (Nottingham, 1982).

2.3.1 Beef carcass contamination

Within the beef slaughter and dressing process, holding pens, carcass skinning and evisceration have been identified as probable introduction points of major contamination (Anon, 1993). Microbial contamination of meat can result in spoilage,

food poisoning and the transmission of certain other diseases (Nottingham, 1964). Contamination can be minimised by good manufacturing processes, but the total elimination of foodborne pathogenic microorganisms is difficult, if not impossible (Dickson and Anderson, 1992).

It is very difficult to obtain clean meat from dirty animals. Therefore the state of the animal at slaughter is important (Nottingham, 1982). Animal sources of carcass contamination include the hide and the gastro-intestinal and respiratory tracts (Ayres, 1955). The cleanliness of the stock depends on a number of factors including location of the farm, method of transport and holding conditions at the processing plant (Nottingham, 1982). When cattle leave the farm or feedlot for the abattoir they will carry within their intestinal tracts and on their hooves and hides a large population of microorganisms (Grau, 1987). Cattle from feedlots may carry more faecal bacteria and fewer soil organisms than cattle from pastures, although faeces from cattle grazed on lush pasture are particularly prone to gross contamination during transport (Nottingham, 1982; Grau, 1979). Contamination on animals from dry dusty areas will differ from that on livestock grazed on pasture under wet muddy conditions (Nottingham, 1982).

Between the farm and the slaughter floor the microbiological status of cattle can change (Grau, 1987). The starving and intermittent feeding that some herds undergo when they travel long distances to slaughter make such animals sensitive to salmonellae (Grau, 1979). With meat processed under hygienic conditions the numbers of pathogens coming from sources outside the animal are usually very small (Nottingham, 1982), and much of the mircoflora transferred to tissue surfaces, while aesthetically undesirable, is nonpathogenic (Dickson and Anderson, 1992).

The significance of contamination during slaughter depends on the numbers of bacteria introduced and the extent to which they are eliminated by the defence mechanisms. The animal's internal carcass tissues can be viewed as being initially sterile but as losing this status with the first dressing procedures.

The methods usually employed for the slaughter of meat animals can provide entry for microorganisms into the tissues (Nottingham, 1982). Slaughter techniques which involve simultaneous severance of oesophagus and cervical blood vessels, can result in

contamination of head and neck meat as a result of ruminal regurgitation (Petersen, 1979).

Both removal and cleaning of the hide and the removal of the gastrointestinal tract and organs requires skilled and careful operators to avoid contamination of edible tissues (Petersen, 1979). Faecal contamination of dressed carcasses can occur as a consequence of direct contact with faeces or contact with surfaces that have themselves been in contact with faeces, e.g. hides (Bell, 1997). In respect to microbial contamination of carcasses, it is not the weight of faeces but the area over which the contamination is spread that will have the most influence on the counts obtained (Bell, 1997). The hands, knives, steels and aprons of the slaughter-persons who handle the carcass before the hide is removed are more often more highly contaminated than those who handle the carcass after the hide is removed (Grau, 1987).

In addition to the skin, the gastro-intestinal and respiratory tracts, urine and milk are other important animal sources of contamination (Nottingham, 1982). The removal of the gastrointestinal tract can cause problems for two main reasons. Firstly, spillage of contents may occur either through the oesophagus or the rectum when these openings are not tied adequately (Petersen, 1979). Secondly, accidental punctures may occur either through knife cuts or when pulling the organs away from the carcass (Petersen, 1979).

Evisceration may have an adverse effect on the contamination of meat (Sheridan, 1998). However, evisceration can be carried out with minimal contamination of the carcass provided the intestinal tract is not ruptured or punctured (Grau, 1979).

Meat receives extensive handling during boning and cutting operations and fresh surfaces are exposed, making it more susceptible to contamination (Nottingham, 1982). The major source of contamination in the boning room is likely to be the incoming meat. The microbial flora on boning room surfaces reflects the microbial flora of the meat passing over it (Grau, 1987). The extent to which this contamination is spread on freshly cut surfaces by contact with work surfaces, equipment and hands can have a major effect on the quality and storage life of the meat (Nottingham, 1982).

The meat handler may also cause indirect cross contamination between carcasses if hands are not cleaned between operations. Aprons and boots should also be cleaned regularly as they may accidentally touch edible products (Petersen, 1979). Indirect cross contamination between carcasses may also be caused by fixed equipment such as stands, platforms, pipes etc (Petersen, 1979).

The surface of a freshly dressed carcass is usually wet and warm, ideal conditions for microbial growth; thus if there is any delay before consumption or further processing it is necessary to cool the carcass (Nottingham, 1982). Contamination can occur during chilling through contact with the walls or floor, by splashing if cleaning is carried out in a loaded chiller and from the air (Nottingham, 1982). For maximum storage life it is essential to prevent the build up of a psychrotrophic flora in the chillers by regular cleaning (Nottingham, 1982). Washed sides of beef are chilled in air to prevent growth of mesophiles and to limit the growth of spoilage organisms (Grau, 1987). Control of growth is achieved by the drying of meat surfaces that takes place during chilling. Sides of beef must be placed in the chiller so that there is no contact between them as contact areas remain warm and moist, and also disturbs airflow (Grau, 1987). Microbial growth is more likely to occur on the thinner, quicker cooling areas of the beef side as these lose less surface moisture. The much larger size of a beef carcass, compared to poultry and sheep, increases the time required to chill it, and so influences the pattern of microbial growth (Grau, 1987). During processing and storage, microbial viability and growth become the principal concerns. Temperature, water activity and oxygen concentration are the major factors affecting microbial growth; carbon dioxide and pH play a lesser role (Nottingham, 1982).

2.3.2 Beef Carcass Decontamination

2.3.2.1 Traditional methods of beef carcass decontamination

To control contamination during processing the basic principle is to aim at the best possible separation between clean and unclean products and between clean and dirty operations (Nottingham, 1964). In recent years the meat industry and regulatory authorities have attempted to limit the presence of pathogens on carcasses by the application of Hazard Analysis and Critical Control Points (HACCP) systems within

meat plants (Sheridan, 1998). These are designed to assist in the management and control of the slaughter process by identifying the critical control points where contamination can occur and by specifying actions that can be taken to improve the hygienic status of the carcass (Sheridan, 1998).

Ante-mortem inspection should prevent the introduction of obviously infected animals to the slaughter floor (Nottingham, 1964). Post- mortem inspection will reject diseased carcasses but not until there has been some opportunity for infection to spread to plant and equipment and to other carcasses (Nottingham, 1964).

Improving skill levels to decrease the possibility of contamination is important especially in areas such as evisceration and hide removal. Tying or sealing the bung (rectum) of animals during slaughter is designed to reduce the spread of faecal material from the rectum to the carcass, while tying off the oesophagus prevents leakage of ingesta. These are critical preliminary operations in controlling contamination during evisceration (Sheridan, 1998). With these sources of carcass contamination effectively contained, further reduction in the contaminating microflora present on beef carcasses depends on reducing the microbial contamination present on the hide/carcass contacts during dressing (Bell, 1997).

Microorganisms of human origin may reach the meat, but contamination can be minimised if attention is paid to sanitation and personal hygiene (Nottingham, 1982). Bell (1997) found that rinsing of operatives' hands between carcasses removed 90% of the hide-derived microflora. Contact between carcass and unrinsed operatives' hands would introduce contamination comparable to hide/carcass contact for those operations in which hide/hand contact is unavoidable (Bell, 1997). Contamination on knife blades was found to be an order of magnitude lower than was found on the operatives' hands (Bell, 1997). Rinse and 82°C water treatment of knives between carcasses effectively reduced bacterial contamination to a low level (Bell, 1997).

Trimming is an on-line process used to remove fat, small faecal spots and smears from beef carcasses (Sheridan, 1998). It is assumed that trimming will completely remove the physical contamination as well as the microbiological contamination of the tissue, assuring the product is safe and wholesome (Reagan *et al.*, 1996). Under commercial

beef slaughtering conditions, trimming may be a highly variable process, with its efficacy primarily related to the skill and/or diligence of the individual doing the trimming (Reagan *et al.*, 1996). An alternative to trimming for the removal of small faecal stains is the use of steam-vacuuming (Sheridan, 1998). An added advantage of steam-vacuuming is the avoidance of producing contaminated waste meat.

Under modern processing conditions, the production of pathogen-free meat cannot be guaranteed. Carcass decontamination refers largely to the use of carcass spray washing systems to reduce and/or kill bacteria on carcasses (Siragusa, 1995). A decontamination step, in the form of washing and sanitising during the slaughter process, can improve the microbial safety and shelf life of the meat and should be considered an integral part of the production process (Dickson and Anderson, 1992). Many HACCP systems recommend pre-evisceration washing or sanitising immediately after dehiding as a means of reducing bacterial counts on the carcass at final dressing (Sheridan, 1998). It is carried out immediately after hide removal in order to obtain maximum effect in terms of bacterial removal. If the carcasses are washed immediately after dehiding the ability of the bacteria to adhere to the meat surface is reduced (Sheridan, 1998).

Physically removing bacteria remaining on carcass surfaces by washing with water and subsequent sanitising has been shown to be a practical and effective means of improving the microbiological quality of fresh meat (Kotula *et al.*, 1974). In a review of decontamination of carcasses, Dickson and Anderson (1992), reported that washing the animal prior to slaughter with cold or hot water, as well as carcass washing, brushing, and drying combinations have all reduced the population of bacteria on carcasses.

Bell (1997) showed that carcass contamination associated with clean hide contact was significantly lower than that following contact with preslaughter washed faecally soiled hide (Bell, 1997). Bell (1997) found that the latter was comparable to that resulting from direct contact with fresh faeces. These observations suggest that washing immediately preslaughter may not be the most efficacious time to address the problem of stock cleanliness. The possibility that carcass washing may potentially spread contamination from one area of a carcass to another must be considered.

However, Reagan *et al.* (1996) found that the current industry practice of trimming and washing of beef carcasses consistently resulted in low bacterial populations and visual scores for faecal contamination. The authors also found that hot-water washing may be an effective intervention strategy in reducing bacteria on beef carcasses, especially in producing more consistently low bacterial populations among carcasses by reducing the carcass-to-carcass variation.

Short chain organic acids have been targeted as the most logical agents to spray on carcasses as antimicrobial agents (Siragusa, 1995). The antimicrobial effect of short chain fatty acids is largely due to the lower pH where the undissociated form of the acid is maintained (Cutter and Siragusa, 1994).

2.3.2.2 Preslaughter methods of reducing carcass contamination

The New Zealand meat industry is based on ruminants - animals with proportionately large digestive tracts that maintain a subtle gastrointestinal balance. Animals, well fed on pasture without a fasting period before transport to slaughter, cause problems during transport and processing due to the composition and large volumes of digesta and faeces. These animals generally have increased faecal material on their hides and skins, which is a risk to meat hygiene. In the case of cattle, a very full digestive tract can be difficult to handle in the meat plant. Furthermore, the volumes of effluent on trucks, and discharge of stockyard waste and digesta into plant effluent streams, contributes greatly to a meat plant's effluent and pollutant loading and costs. To reduce these problems, farmers are generally encouraged to fast their animals for a period before transport. Fasting decreases rumen and intestine weight (Bass and Duganzich, 1980) and fasted animals produce less faecal waste on stock trucks. However, fasting cattle for periods of about 20 hours or more can cause dehydration and carcass weight loss (Jacobson *et al.*, 1998). Fasting also decreases the dry matter content and particle size of digesta, which increases the risk to meat hygiene. Feeding cattle with hay or other conserved forages may increase dry matter of the digesta thereby decreasing the risk of carcass contamination at slaughter.

2.3.3 Poultry carcass contamination

Consumers have been cautioned repeatedly about the microbiological safety of the poultry and poultry products they consume (Kotula and Pandya, 1995). Microorganisms normally found on poultry are also associated with other animals. Pathogens of particular concern on poultry carcasses include *Salmonella* spp., *Campylobacter jejuni/coli*, and *Escherichia coli* (Kotula and Pandya, 1995). The microbiological condition of the processed carcass is related to the numbers and types of microorganisms carried into the processing plant by the live bird and to the effectiveness of control measures within the plant (Mead, 1982).

Poultry processing is highly intensive in the larger slaughterhouses, with production rates of 6000 or more carcasses per hour on a single line and birds virtually touching each other on the processing line (Bremner and Johnston, 1996). This situation is particularly conducive to microbial cross-contamination, allowing little opportunity to sanitise implements or equipment between carcasses (Mead, 1982; Bremner and Johnston, 1996). During conversion of chicken into meat for consumption, the carcasses pass though various stages of processing (Sakhare *et al.*, 1999). There is every opportunity for increasing microbial numbers on carcasses during processing, due to cross contamination from the processing environment, from other birds and from the processing equipment. Contamination of carcasses may originate from bacteria carried on the skin or feathers of the birds, from spillage of intestinal or crop contents during evisceration, or from cross-contamination from sources within the plant (Renwick *et al.*, 1993). The carcass must be kept whole throughout the entire processing operation so that the viscera have to be removed rapidly through a small vent and as far as possible without breakage if contamination of the carcass with intestinal organisms is to be minimised (Mead, 1982).

Part of the problem for the processor is that the birds arriving for slaughter are heavily contaminated with a wide variety of micro-organisms, which are carried in the alimentary tract, among the feathers and on the skin (Bremner and Johnston, 1996). Renwick *et al.* (1993) found that bacterial load increased significantly as the mean visible contamination score increased. This would be expected since contamination from faeces or ingesta should logically increase the total bacterial load on chickens. It

is normal practice to withhold feed for several hours at least to reduce the natural distension of the intestines and therefore minimise gut breakage during processing (Bremner and Johnston, 1996). Despite this precaution, however, there is still a spread of faecal material during the period of transfer from farm to processing plant, and a parallel increase in microbial contamination of skin and feathers (Bremner and Johnston, 1996). Carcass bacterial load increases with increases in crating and holding time, visible contamination, and outdoor temperatures (Renwick *et al.*, 1993). Transport crate and vehicles can be a potent source of flock-to-flock transmission for enteric food-borne pathogens, and need to be properly cleaned and disinfected after delivering birds to the processing plant (Bremner and Johnston, 1996). The different stages of processing result in many of these organisms being removed, although further contamination is likely as birds pass through the process (Bremner and Johnston, 1996).

Lillard (1990) determined the levels of aerobic bacteria, *Enterobacteriaceae*, and *Salmonella* at six sampling points in a commercial processing plant: (1) pre-scald (at bleed line); (2) post-scald; (3) post-pick; (4) post-evisceration; (5) pre-chill (after final washer); and (6) post-chill. He found that the level of aerobic bacteria and *Enterobacteriaceae* on broiler carcasses was reduced significantly by commercial processing procedures, but cross-contamination still occurred.

Kotula and Pandya (1995) found that broilers arriving at the processing plants were highly contaminated with potential human pathogenic bacteria (*E. coli*, *Salmonella* spp., and *C. jejuni/coli*) on the feathers, skin and feet. They found that the feathers were more highly contaminated than skin or feet.

The scalding operation generally increases the microbial load in scald water (Sakhare *et al.*, 1999). It is necessary to reduce the build up of microbial load in scald water to reduce the cross contamination of carcasses (Sakhare *et al.*, 1999).

Though the feathers and feet are removed during processing, there is ample opportunity during grow-out for microorganisms from the feathers and feet to be transferred to the skin of the carcass, which is a consumable portion (Kotula and Pandya, 1995). Defeathering is generally considered to be one of the major causes of cross contamination early in the processing (Sakhare *et al.*, 1999). The plucking process has

important microbiological implications. The scouring or flailing of carcasses with a large number of flexible rubber 'fingers' causes widespread dissemination of microorganisms (Bremner and Johnstone, 1996).

As a result of removal of the epidermal layer by the plucking process, the exposed tissue provides a new surface for colonisation of bacteria (Thomas and McMeekin, 1980). Following mechanical defeathering, in itself a process which tends to scatter micro-organisms, the skin is left with many holes which are capable of trapping contaminants during the remainder of the processing operation (Mead, 1982).

Lillard (1989) found that broilers coming into a processing plant at the preslaughter stage already have bacteria attached to the skin, and that all bacteria are not removed by a single, or even by 10 consecutive rinses. Aerobic bacteria and *Enterobacteriaceae* were found to be firmly attached to broilers at the pre-scald and post picking stages before evisceration was initiated, and persisted through various points in processing (Lillard, 1989).

There was a significant increase in *Salmonella* incidence on carcasses exiting the immersion chiller, indicating that this may be the point of most significant cross-contamination in broiler processing plants (Lillard, 1990).

2.3.4 Poultry Carcass Decontamination

2.3.4.1 Traditional methods of poultry decontamination

Proper sanitation, holding temperatures, and cooking, along with avoidance of cross-contamination, are control procedures that can ensure the safety of the food that we eat (Kotula and Pandya, 1995). Decontamination is a bactericidal treatment applied to reduce pathogenic and spoilage organisms. Scald tanks and feather pickers need to be kept clean to minimise cross-contamination of the skin by bacteria on the feathers of the birds (Kotula and Pandya, 1995). By decontaminating at every step, hygienic meat production should aim at reducing the microbial contamination of the carcasses at each stage of processing (Sakhare, 1999).

Rinsing carcasses with water is suggested to be the effective means for reducing the microbial load attached to carcass surfaces (Siragusa, 1995). Carcass washing reduces the population of bacteria on carcasses by removal of liquid film containing microorganisms before they become more closely attached with the skin surface.

Spray washing replaces the contaminated water film on carcasses with a clean film of water, thus reducing the microbial load (Mulder, 1985). Sakhare *et al.* (1999) found that repeated spray washing throughout poultry processing with dilute acid (acetic or lactic) is more effective than spray washing with water alone.

Several organic acids such as lactic acid and acetic acid have been suggested as decontaminating agents for poultry (Sakhare, 1999). Organic acids are required at higher concentrations to be effective as decontaminating agents, but it is important to consider the effect of high concentrations of acids on product quality (Siragusa, 1995). Sakhare *et al.* (1999) found that the addition of acetic or lactic acid did not affect the total aerobic bacterial count in scald water before the immersion of birds, but scalding of birds in water containing lactic acid reduced the microbial load on the carcasses. They found the addition of lactic acid to scald water would reduce the microbial load in scald water and thus reduce the cross contamination of carcasses from scald water. Scalding of the birds in acidified water or spray washing of the carcasses after scalding, with water containing acid reduces the microbial load on the carcasses (Sakhare *et al.*, 1999).

2.3.4.2 Preslaughter methods of reducing carcass contamination

Kotula and Pandya (1995) suggested on the basis of the high bacterial incidence and counts on broiler feathers, skin and feet upon arrival at the processing plant there is great need to determine methods to decrease microbial load and incidence on birds entering the processing plant. There is a need to further decrease the microbial load and incidence as carcasses travel through the plant (Kotula and Pandya, 1995). One of the newest and most useful means of controlling *Salmonella* infection in poultry, in combination with conventional hygienic measures, and one which can be applied to commercial poultry flocks, is microbial colonisation control (Anon, 1993). This is known as competitive exclusion, which prevents colonisation by enteropathogens

(Anon, 1993). Kotula and Pandya (1995) suggested that sanitation of grow-out facilities and conditions of transport are extremely important considerations in reducing broiler contamination with potentially pathogenic bacteria.

Reduction of the numbers of potential pathogens entering the processing plant in or on animals is dependent on the ability to influence the environmental conditions of the gastro-intestinal tract. In order to achieve this an understanding of the factors influencing gut flora is needed.

2.4 FACTORS INFLUENCING GUT FLORA

The micro-organisms which finally come to rest in the gut are those best fitted for survival, and are selected from the huge range of microbes in the external environment (Clarke, 1977). Four main conditions influence the physical environment of a microbe: temperature, pH, gaseous atmosphere, and osmotic pressure (Pelczar *et al.*, 1993). In order to survive, the organism must be able to tolerate the physical conditions of the particular gut environment, obtain sufficient materials for its nutritive needs, compete successfully with other organisms for the niche, and multiply at a rate sufficient to avoid being washed out (Clarke, 1977).

2.4.1 Temperature

Temperature is one of the most important variables affecting the growth of micro-organisms (Clarke, 1977; Pelczar *et al.*, 1993). All the processes of growth are dependent on chemical reactions that are affected by temperature (Pelczar *et al.*, 1993). In addition to influencing the rate of cellular reactions, temperature affects the microbial environment. Factors affected include: pH value, water activity, ion activity, viscosity, hydration and aggregation of macromolecules, toxic action of metabolic products and other unfavourable components of the medium, solubility of gases (especially oxygen and carbon dioxide) (Clarke, 1977).

Microorganisms can grow over a broad range of temperatures. At the most favourable temperatures for growth, the number of cell divisions per hour (the growth rate) generally doubles for every temperature increase of 10°C (Pelczar *et al.*, 1993). The

temperature at which a species of microorganism grows most rapidly is the optimum growth temperature. For any particular microbe, the three important temperatures are the minimum, optimum and maximum growth temperatures (Pelczar *et al.*, 1993). These are known as the cardinal temperatures of a species of microorganism and may vary with the stage in the life cycle of the microorganism (Pelczar *et al.*, 1993).

In addition to affecting the growth rate, temperature may also affect the type of reproduction, morphology, metabolic processes, and nutritional requirements. The optimum temperature for a microbial species is nearer the upper limit of the temperature range, because the rate of enzyme reactions increases with increasing temperature until a point where the enzymes are damaged by heat and cells stop growing (Pelczar *et al.*, 1993).

Microorganisms may be divided into three groups on the basis of the temperature range in which they grow best:

1. Psychrophiles, or cold loving microbes
2. Mesophiles, or moderate temperature loving microbes
3. Thermophiles, or heat-loving microbes.

Most microorganisms are mesophiles, growing best within a temperature range of 25 to 40°C (Pelczar *et al.*, 1993). Parasitic organisms of humans and animals grow in the upper part of this range. Those that are pathogenic for humans grow best at about body temperature, which is 37°C.

Heat evolved during fermentation may also be of value in maintaining the body temperature of warm-blooded animals in cold climates (Hungate, 1966). This would apply particularly to herbivores with their large mass of fermenting digesta. The temperature in the rumen of sheep and cows is 39-40°C (Hungate, 1966) but may be as high as 41° during active fermentation (Clarke, 1977). In monogastic animals the effect of ingestion of food or water on the temperature of the lower gut, and on microbial activity is negligible (Clarke, 1977).

2.4.2 pH

Microorganisms have certain pH (hydrogen ion concentration) needs (Wistreich and Lechtman, 1988). In contrast to optimum temperature, the optimum pH for microbial growth lies approximately in the middle of the pH range. The optimum pH is usually well defined for individual species; different species are adapted to grow at various pH values (Pelczar *et al.*, 1993). For most bacteria, the pH minimum is about 4, with pH 9 as the maximum for growth (Pelczar *et al.*, 1993). As a rule, microorganisms prefer a more neutral pH, between 6 and 7.5 (Wistreich and Lechtman, 1988) and the areas of the gut where microorganisms are found in high numbers usually have a pH between 5.0 and 7.5 (Clarke, 1977).

To grow well in an acidic or a basic environment, a microorganism must be able to maintain its intracellular pH at about 7.5, regardless of the external pH (Pelczar *et al.*, 1993). A living cell has the ability, within limits, to keep a constant internal pH by expelling hydrogen ions or by taking hydrogen ions into the cell (Pelczar *et al.*, 1993). Thus the pH of its external environment usually has to change drastically before the inside of the cell is affected.

The stomachs of simple-stomached animals generally do not support microbial growth because of the high acidity of the contents. In all vertebrates, the true stomach acts as a barrier to microorganisms reaching the small intestine (Clarke, 1977). The abomasum, the acid-secreting stomach of ruminants, maintains a hydrogen ion concentration sufficient to destroy most of the microorganisms entering from the rumen and omasum (Hungate, 1966). In regions of the gut where high numbers of microbes are found, such as the rumen and caecum, acidity is influenced by products of microbial fermentation, particularly the volatile fatty acids (VFA), and bicarbonate and phosphate secreted by the host (Clarke, 1977).

2.4.3 Gas Composition

Microorganisms in their natural habitats require varying amounts of gases such as oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2) and methane (CH_4) (Pelczar *et al.*, 1993). Gases in the gut, apart from N_2 and O_2 are of microbial origin. The composition

of the gas mixture varies with animal and region of the gut. Swallowed air is the main source of N_2 and O_2, but this influences the gas phase only in the upper regions of the alimentary tract; the stomach in non-ruminant vertebrates and the rumen in ruminants (Clarke, 1977).

Carbon dioxide and oxygen are the two principal gases that affect the growth of microbial cells (Pelczar *et al.*, 1993). Microorganisms react in varying degrees to O_2 (Clarke, 1977). All cells for certain chemical reactions use carbon dioxide; however, oxygen is required by some microorganisms but is toxic to others (Pelczar *et al.*, 1993). On the basis of their response to gaseous oxygen, microorganisms are divided into four physiological groups: aerobes, facultative microorganisms, anaerobes, and microaerophiles.

Aerobic microorganisms are microbes that normally require oxygen for growth and can grow in a standard air atmosphere of 21% oxygen (Pelczar *et al.*, 1993). Facultative microorganisms are those that grow in an air atmosphere and can also grow anaerobically (Clarke. 1977; Pelczar *et al.*, 1993). They do not require oxygen for growth, although they may use it for energy-yielding chemical reactions. Anaerobic microorganisms are those that may be poisoned by oxygen, cannot grow in an air atmosphere, and do not use oxygen for energy yielding chemical reactions (Pelczar *et al.*, 1993). Microaerophiles are organisms, which like aerobes can use oxygen for energy-yielding chemical reactions. However, unlike aerobes, they cannot withstand the level of oxygen (21%) present in an air atmosphere and usually grow best at oxygen levels between 1 and 15% (Pelczar *et al.*, 1993).

In the rumen the gas mixture is approximately 63% CO_2, 27% CH_4, 7% N_2 with traces of H_2 and H_2S and with small transient amounts of O_2 (Clarke, 1977). Methane is produced in the lower gut of herbivores as well as in the rumen.

2.4.4 Osmotic and Ionic Effects

Bacterial growth can be influenced by the force or tension built up when water diffuses through cell membranes (Wistreich and Lechtman, 1988). The force that drives the water is known as osmotic pressure and results from the tendency of water molecules to

pass through the membrane and to equalise the concentration on both sides of the membrane. When a cell is placed into a fluid environment that has exactly the same osmotic pressure as the cell, there is no movement of water molecules either in or out of the cell. The cell neither swells nor shrinks and the surrounding fluid is said to be isotonic (Wistreich and Lechtman, 1988). Bacterial cells placed into a fluid having a lower osmotic pressure will swell because water enters the cells. The surrounding fluid is said to be hypotonic. In most cases the rigid cell walls of bacteria, fungi, algae, and plants enable these cells to withstand an external fluid environment that is hypotonic without bursting (Wistreich and Lechtman, 1988).

When cells with walls are placed into a (hypertonic) solution having a greater concentration of dissolved materials, water passes out into the surrounding environment and the contents of the cell shrink (Wistreich and Lechtman, 1988).

Most bacteria are resistant to a considerable degree of osmotic shock and are able to remain osmotically balanced with their environment. The osmolality of gut contents varies with the type of food eaten and the region of the gut (Clarke, 1977). The osmolality of rumen contents may drop markedly after water is drunk and require several hours to recover (Clarke, 1977).

2.4.5 Other factors influencing gut flora

2.4.5.1 Surface Tension

Surface tension can markedly affect microbial cell physiology, some of the effects being stimulatory to growth and some inhibitory (Clarke, 1977).

2.4.5.2 Nutrients and Liquid Flow

Rates of flow of digesta vary in different animals depending on their anatomy, physiology, and dietary habits and requirements. The success of an organism in any gut environment depends on its ability to grow on the nutrients available, at a rate high enough to avoid being washed out (Clarke, 1977). In herbivores the digesta is retained for considerable periods in the modified forestomachs or in the enlarged large intestine

and a dense, complex population of microorganisms develops (Clarke, 1977). In the ruminant these microorganisms ferment the ingested food but in the caecal fermentation the microorganisms utilise what remains after modification in the true stomach and digestion and absorption in the small intestine (Clarke, 1977).

2.4.5.3 Competition

If two organisms in a continuous culture system have closely overlapping preferences for a growth-limiting substrate, they will compete severely for the common nutrient source, and the inferior organism will be excluded (Lee, 1985). If they have different preferences, such as the degree of overlapping is less, they may coexist, since these organisms will then occupy different ecological niches (Lee, 1985).

2.4.5.4 Substrate

A large diversity of microbes may be present in the gut. This is a consequence of the variety of chemical compounds available as substrates (Clarke, 1977). Gut bacteria contribute to a widening range of available substrates by their own degradative action, thus increasing the opportunity for more species to survive (Lee, 1985). The substrates available for fermentation are prime factors in determining the microbial population in various regions of the gut (Clarke, 1977). Innumerable microbial interrelationships have been found in the gut where one species utilises materials excreted by other species (Lee, 1985).

2.4.6 Rumen flora

For the microorganisms that have taken up residence in the rumen, the situation offers advantages; they are provided with an environment always rich in fermentable carbohydrates, well buffered by the saliva, and maintained at a constant favourable temperature, the body temperature of the cow (Stanier *et al.*, 1970). The rumen bacteria are adapted to live in acidities between pH5.5 and 7.0, in the absence of oxygen, at a temperature of 39-40° C, in the presence of moderate concentration of fermentation products, and at the expense of the ingesta provided by the ruminant (Hungate, 1966).

The predominant microbes in the rumen are anaerobic bacteria, protozoa and fungi (Duncan *et al.*, 1999). Microbial interactions within the rumen are complex, intensely competitive and are known to inhibit the growth of enterobacteria (Rasmussen *et al.*, 1999). Although facultatively anaerobic enterobacteria are present in the rumen, they usually occur at relatively low population densities and are generally regarded as transient and non-growing (Duncan *et al.*, 1999; Rasmussen *et al.*, 1999).

The predominance of strict anaerobes and the inhibitory relationship between pH and the volatile fatty acids produced during fermentation have led to *E. coli* being thought of as unimportant in rumen microbiota (Rasmussen *et al.*, 1999). These microbes were considered poor competitors in relation to the predominant population of strict anaerobes found in this environment (Rasmussen *et al.*, 1999).

Brown *et al.* (1997) found that within the gastrointestinal tract, the forestomachs were a primary site of *E. coli* O157:H7 localisation and proliferation. *E. coli* O157:H7 was consistently isolated from rumen fluid and from the rumen and omasum at necropsy in all affected calves (Brown *et al.*, 1997). Rumen inhibition and the variability in faecal shedding raise questions concerning the nature of the bovine reservoir and whether a 'true carrier state' exists (Rasmussen *et al.*, 1999). Factors, which influence the proliferation of E. coli O157:H7 in the forestomachs, may then ultimately determine the magnitude of faecal shedding (Brown *et al.*, 1997). Rasmussen *et al.* (1999) suggested that individuals can carry and clear *E. coli* O157:H7 within a relatively brief period of time. Brown *et al.* (1997) found that in gastrointestinal sites, *E. coli* O157:H7 appears to localise within the intestinal contents rather than colonise the mucosal surface; therefore the importance of preslaughter feeding cannot be underestimated.

2.4.7 Poultry flora

An important consideration is the present practice in the modern poultry industry of rearing chickens away from adult birds and in hygienic conditions where there is little opportunity for the chicken to acquire an indigenous intestinal flora (Lloyd *et al.*, 1977).

The incubator-hatched chicken is an example of an animal that has been deprived of its characteristic protective gut microflora by the artificial environment imposed upon it

(Fuller, 1999). It emerges from the egg into a clean hatchery totally removed from any contact with its mother or any adult chickens. It therefore acquires its gut microflora from its immediate environment, which does not contain all the microorganisms required to confer protection on the newly hatched chick (Fuller, 1999). Modern hygienic practices in poultry sheds, while admirable for control of many poultry pathogens, may result in a delay in the development of a stable indigenous flora of the intestine (Nurmi and Rantala, 1973; Lloyd *et al.*, 1977). This lack of indigenous flora could deny the host part of its natural defence mechanism against opportunistic enteric pathogens. There is increasing interest in the possibilities of manipulating the composition of the gut microflora by foods or food ingredients.

2.5 MANIPULATION OF GUT FLORA IN THE GROWING ENVIRONMENT OR PRESLAUGHTER PERIOD

With an understanding of the factors influencing the microflora of the gastrointestinal tract, manipulation of these factors and therefore the composition of the gut flora can be undertaken. Gut flora in animals can be manipulated through various methods. These include varying the volatile fatty acid concentration (VFA), withholding feed, providing different varieties of feed, including compounds in feeds such as ionophores, probiotics, prebiotics and synbioitcs, and controlling stress.

2.5.1 Ruminant Digestion and Volatile Fatty Acids

The digestive tract of a ruminant contains no less than four successive stomachs. The first two, known as the rumen and reticulum, are essentially vast incubation chambers teeming with bacteria and protozoa (Stanier *et al.*, 1970). A cooperative relationship predominates in herbivores whose food contains abundant plant material high in carbohydrates indigestible by mammalian enzymes (Hungate, 1978). The plant materials ingested by the cow are mixed with a copious amount of saliva and then passed in to the rumen where they are rapidly attacked by bacteria and protozoa (Stanier *et al.*, 1970).

The microbial fermentation chamber precedes the acid stomach. It is kept at a near neutral pH, and is populated by myriads of microbes growing in the absence of oxygen

and fermenting carbohydrates to form new cells and giving off CO_2, H_2 or CH_4, and short-chain volatile fatty acids as waste products (Hungate, 1978). The cellulose and other complex carbohydrates present in the ingested fodder are broken down with the eventual formation of simple fatty acids (acetic, propionic and butyric) and gases (carbon dioxide and methane) (Stanier *et al.*, 1970). The anaerobic bacteria, protozoa and fungi in the rumen produce volatile fatty acids (VFA) from the carbohydrate polymers present in the diet (Duncan *et al.*, 2000). Ruminal bacteria have adapted to volatile fatty acid concentrations that are usually greater that 100mM (Russell and Diez-Gonzalez, 1998). The fatty acids are absorbed through the wall of the rumen into the blood stream, circulating in the blood to the various tissues of the body where they are metabolised (Stanier *et al.*, 1970).

2.5.2 Volatile Fatty Acids and Diet

Dietary changes can influence the concentrations of VFA in the rumen and the colon, thereby affecting the pH of the gut contents (Duncan *et al.*, 2000). The pH and concentrations of VFA are related to the frequency of feeding and may fluctuate markedly in relation to the elapsed time after feeding (Duncan *et al.*, 2000). To increase VFA concentrations by feeding bulk solids, the substrates required, such as plant cell-wall polysaccharides, are those that normally make up the bulk of the ruminant diet (Duncan *et al.*, 1999). This approach equates to maintenance of feed supply during the period prior to slaughter. VFA concentrations and pH may remain relatively constant in animals on a continuous feeding regime (Duncan *et al.*, 2000).

2.5.3 Volatile Fatty Acids and *E. coli*

The occurrence of relatively low numbers of *E. coli* in the ruminant gut has been ascribed to the inhibitory effects of VFAs (Rasmussen *et al.*, 1993). An unfavourable environment for survival of *E. coli* can be created through elevation of VFA (Duncan *et al.*, 1999). There are two ways to approach this:

1. Through direct supplementation of the diet with VFA, or
2. By stimulating VFA production from commensal anaerobes through supply of appropriate substrates that are unavailable to *E. coli*.

Wolin (1969) reported that there would seem little doubt that volatile fatty acids could play an important role in excluding *E. coli* and other volatile fatty acid-sensitive organisms from the rumen. Since the pH of rumen contents usually is in the range of 6.0 to 7.0, however, it is probable that toxic levels of nonionised volatile fatty acids are present only between pH 6.0 to 6.5 (Wolin, 1969).

Fasting might be expected to cause the proliferation of *E. coli* O157:H7 in the rumen or colon, because rumen pH increases and total VFA concentrations decrease (Harmon *et al.*, 1999). Harmon *et al.* (1999) believe acid-tolerant *E. coli* O157:H7 may not be sensitive to the fluctuations in VFA concentrations of the rumen and colon, as are other coliforms. Rasmussen *et al.* (1993) found for strains of O157:H7 that a clear tendency existed for growth rate to decline with increasing volatile fatty acids and decreasing pH. Animals fed roughage might be expected to shed higher populations of *E. coli* O157:H7, due to consistently lower VFA concentrations when compared with grain-fed calves (Harmon *et al.*, 1999).

2.5.4 Fasting and *E. coli*

All strains of *E. coli* show unrestricted growth in rumen fluid collected from fasted cattle, thus indicating a role for fasting in the ecology of *E. coli* in cattle (Rasmussen *et al.*, 1993). The effects upon the population dynamics of *E. coli* O157:H7 have not been as pronounced as that observed for other strains of the microbe. Harmon *et al.* (1999) found fasting cattle results in the proliferation of coliforms in the bovine rumen and subsequent increases in faecal shedding.

A combination of fasting and sporadic feeding perturbed normal gut microflora to such as extent that minor population members like *E. coli* could temporarily predominate in this environment (Rasmussen *et al.*, 1999). Feed withdrawal can result in 3×10^4 and 10^7 fold increases in ruminal *Salmonella* sp. and *E. coli* populations, respectively (Brownlie and Grau, 1967). At abattoirs, cattle that are held for longer periods without feed tend to have larger populations of *E. coli* and *Salmonella* sp. in the rumen when slaughtered (Grau and Brownlie, 1968). Hancock *et al.* (1997) agree and found through an epidemiological study of the prevalence of *E. coli* O157:H7 in feedlot cattle, a threefold higher increase in cattle that had been on feed for the shortest time.

Other research has shown that fasting has little or no effects on the proliferation of *E. coli* O157:H7 in the gastro-intestinal tract. Cray *et al.* (1998) found no significant increase in faecal shedding of strain O157:H7 as a result of fasting. However, fasting did make calves more susceptible to a given inoculation dose. Harmon *et al.* (1999) compared faecal shedding of *E. coli* O157:H7, in calves before, during and after fasting periods. The authors found that there was a dramatic decrease in rumen VFA concentration and a corresponding increase in rumen pH, however no consistent difference in the size of the populations of *E. coli* O157:H7 shed on fasting days or on days following the resumption of feeding.

Brown *et al.* (1997) could not find a clear association between fasting and increased faecal shedding of *E. coli* O157:H7 in calves experimentally inoculated with *E. coli* O157:H7. Animals were fasted at a time when the numbers of *E. coli* O157:H7 bacteria in the faeces were decreasing, therefore the reduction of *E. coli* O157:H7 numbers after fasting seen in some calves may have been less than if fasting had not occurred (Brown *et al.*, 1997). The authors found that the most consistent increase in shedding of *E. coli* O157:H7 after fasting was observed in calves shedding low numbers of *E. coli* O157:H7 at the time the fast was instituted.

2.5.5 Feed Type and *E. coli*

In an animal on a predominantly hay or forage ration the majority of the bacteria are gram-negative (Hungate, 1966). With high grain rations, there is an increased proportion of gram-positive cells (Hungate, 1966). The effect of feed composition and feed additives on rumen proliferation and faecal shedding of *E. coli* O157:H7 is largely unknown. However, with well-fed cattle a variety of *E. coli* strains, including strains of O157:H7, were inhibited by the prevailing pH and volatile fatty acid concentrations of the rumen (Rasmussen *et al.*, 1993).

Diez-Gonzalez *et al.* (1998) have suggested that grain creates an acidic environment in the gut of the cattle and this in turn leads to the selection of acid-tolerant *E. coli*, including O157 strains, which are then heavily shed in the faeces. The authors proposed that cattle producers could solve the *E. coli* problem by feeding hay to cattle before

slaughter. Harmon *et al* (1999) reported the results of experiments involving the experimental infection of calves receiving a diet high in roughage (Bermuda hay) and calves fed primarily grain. Faecal shedding was highly variable, but the number of *E. coli* O157 shed was, on average, approximately 10-fold higher in the grain-fed animals than in those fed hay. Steers fed hay were found to shed experimentally introduced *E. coli* O157 longer than steers fed grain (Hovde *et al.*, 1999). Brownlie and Grau (1967) found that when cattle received a regular ration of 6.8kg of lucerne hay, *E. coli* was rapidly eliminated from the rumen. Decreasing the food intake or an interruption of feeding for one or more days permitted the growth of *E. coli* in the rumen. Hancock *et al.* (1994) disagreed and found no difference in O157:H7 prevalence between grain fed and hay fed cattle.

Jordan and McEwen (1998) found that cattle on a high-roughage ration for four days had a significantly lower initial log *E. coli* CFU/g of faeces compared to cattle on a normal ration, but after 48 hours of fasting they had a significantly higher concentration. The high-roughage ration fed for 4 days before slaughter was successful in creating significantly lower initial *E. coli* concentrations in the faeces, this advantage was lost by 24 hours of fasting and was reversed by 48 hours of fasting (Jordan and McEwen, 1998). High-roughage rations tend to persist for longer in the rumen but do not reduce the rumen pH as much as high-energy rations (Jordan and McEwen, 1998).

Dargatz *et al.* (1997) examined faecal samples from cattle from 100 feedlots in 13 American states and showed that feeding barley increased the incidence of faecal contamination, while feeding soy meal decreased the incidence of detection of EHEC in faecal samples. Herriott *et al.* (1998) evaluated management practices in 36 Pacific Northwest diary herds and their association with Shiga toxin-positive *Escherichia coli* O157 and found a significantly higher prevalence of *E. coli* O157 in herds fed corn silage than those not fed corn silage.

2.5.6 Compounds in Feeds and *E. coli*

Feeding forage legumes has been reported to decrease shedding of *E. coli*; these plants are especially rich in plant secondary compounds, including coumarins (Murray *et al.*, 1982). It has been shown that *E. coli* O157 strain 12900 was inhibited by coumarin

aglycones, such as aesculetin, umbellifferone, coumarin and scopoletin, under both aerobic and anaerobic conditions *in vitro* (Duncan *et al.*, 1998). Gossypol, a compound that is found in cottonseed, is known to inhibit the growth of a wide variety of Gram-positive bacteria (Rasmussen *et al.*, 1999). Rasmussen *et al.* (1999) have found that gossypol had little effect upon the growth of *E. coli*.

2.5.7 Ionophores and *E. coli*

The use of ionophores has attracted interest, given the apparent temporal relationship between initial ionophore use in the US cattle industry (1970s) and the increase in *E. coli* O157:H7 cases (Rasmussen *et al.*, 1999). However, experimentation in cattle has not been able to establish a direct cause and effect relationship between ionophore use and *E. coli* O157:H7 shedding (Rasmussen *et al.*, 1999).

2.5.8 Probiotics, Prebiotics and Synbiotics

A probiotic is a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). The incubator-hatched chicken is an example of an animal which has been deprived of its characteristic protective gut microflora by the artificial environment imposed upon it (Fuller, 1999). It emerges from the egg into a clean hatchery totally removed from any contact with its mother or any adult chickens. It therefore acquires its gut microflora from its immediate environment which does not contain all the microorganisms required to confer protection on the newly hatched chick (Fuller, 1999). As a consequence it is more susceptible to colonisation of the gut with salmonella that can pose a public health risk by contaminating carcasses (Fuller, 1999).

There is increasing interest in the possibilities of manipulating the composition of the gut microflora by foods or food ingredients. Attempts have been made to improve health status by modulating the indigenous intestinal flora by live microbial adjuncts, called probiotics (Holzapfel, 1998). Two separate approaches exist to increase the number of health-promoting organisms in the gastrointestinal tract.

The first is the oral administration of live beneficial microbes, termed probiotics. Probiotics are defined as mono- or mixed cultures of live microorganisms which, when applied to animal or humans, beneficially affect the host by improving the properties of the indigenous microflora (Fuller, 1989; Holzapfel, 1998; Walker and Duffy, 1998, Crittenden, 1999; Short and Merryfield, 1999). Survival of probiotic organisms in the gut depends on their possessing colonisation factors which enable them to resist the antibacterial mechanisms (chemical and physical) which operate in the gut (Fuller, 1989).

The second is to selectively modify the composition of the microflora using dietary supplements called prebiotics. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health (Walker and Duffy, 1998; Short and Merryfield, 1999; Crittenden, 1999). Prebiotics promote the proliferation of bifidobacteria and endogenous lactic acid bacteria in the colon (Crittenden, 1999; Walker and Duffy, 1998) and can potentially modulate lipid metabolism (Walker and Duffy, 1998).

The concept of 'synbiotic' has been proposed to characterise colonic foods with prebiotic and probiotic properties as health enhancing functional foods (Walker and Duffy, 1998).

Protective flora which become established in the gut are very stable but can be influenced by excessive hygiene, antibiotic therapy and stress (Fuller, 1989). Major factors affecting the microflora of the gastrointestinal tract are:

1. Host mediated factors, such as pH, secretions (immunoglobins, bile, salts, enzymes), motility and physiology
2. Microbial factors, such as adhesion, motility, nutritional flexibility, spores, capsules, enzymes, antimicrobial components and generation time
3. Microbial interactions, such as synergy and antagonism/stimulation
4. Diet, such as composition, non-digestible fibres and drugs (Holzapfel, 1998).

There are four possible mechanisms by which probiotic and prebiotic supplements affect microecology of the intestinal tract. Antibacterial agents (bacteriocins) that are

produced or secreted by probiotic organisms may have an inhibitory effect on pathogenic microflora (Walker and Duffy, 1998). Human breast milk may alter bacterial antagonism for essential nutrients and impede overgrowth of aerobes (Walker and Duffy, 1998). Stimulation of immune responses may suppress potential pathogens, and specific competition for adhesion receptors to gut epithelium may allow lactic acid bacteria and bifidobacteria to occupy the niche normally required by toxin producing organisms for colonisation (Walker and Duffy, 1998).

Potential beneficial effects of probiotics for farm animals include greater resistance to infectious disease, increased growth rate, improved feed conversion, improved digestion, better absorption of nutrients, provision of essential nutrients, increased milk yield, improved milk quality, increased egg production, improved egg quality, improved carcass quality and less contamination (Fuller, 1999).

The prebiotic approach to increasing beneficial bacteria in the colon potentially provides some advantages over the probiotic strategy. Consumed probiotic bacteria must survive transit through the hostile conditions in the stomach and then adapt quickly to their new environment (Crittenden, 1999). In the colon they must compete for nutrients and colonisation sites against an established microflora with species that already occupy the available physical and metabolic niches (Crittenden, 1999). In contrast to probiotics, prebiotics target commensal bacteria specific to the host, and with effective colonisation sites (Crittenden, 1999). Prebiotics offer not only the potential to increase the numbers of beneficial bacteria, but also their metabolic activity through the supply of a fermentable substrate (Crittenden, 1999).

The lactic acid bacteria are still the most commonly used probiotic microorganisms. They are attractive because they are known to be involved in control of the gut microflora and because they are non-pathogenic (Fuller, 1993). In adult animals, lactic acid producing bacteria, often the main constituents of 'probiotic' preparations, comprise such a small proportion of the gut microbial community that a protective role based on direct inhibition of the growth of pathogens is questionable (Duncan *et al.*, 2000). Among facultative or microaerophilic gut bacteria other than *E. coli*, the lactic-acid producing bacteria are often credited with a protective role against pathogens, largely based on the antimicrobial effects of lactic acid (Duncan *et al.*, 2000).

2.5.8.1 Probiotics and E. coli

One possible approach to reducing faecal shedding of *E. coli* O157:H7 in cattle is the use of probiotic bacteria. Introduction of bovine commensal *E. coli* that colonise the same anatomic locations as *E. coli* O157:H7 and produce toxic metabolites, such as colicins, may reduce the carriage of *E. coli* O157:H7 in the bovine digestive tract (Harmon *et al.*, 1999).

Zhao *et al.* (1998) compared faecal shedding and rumen proliferation of *E. coli* O157:H7 in calves fed probiotic bacteria with control calves fed only *E. coli* O157:H7. The authors found that five of the six calves treated with probiotic bacteria stopped shedding *E. coli* O157:H7 before the nine control calves. Both rumen and faecal cultures from these five probiotic calves became *E. coli* O157:H7 negative within 18 days, whereas only one of the probiotic-treated calves and all of the control calves continued to shed *E. coli* O157:H7 until the study was terminated (Zhao *et al.*, 1998). Treatment of cattle with probiotic bacteria can reduce the level of carriage and faecal shedding of *E. coli* O157:H7 and may thereby reduce environmental contamination with this pathogen (Zhao *et al.*, 1998).

2.5.9 Stress and *E. coli*

When normal fermentation is disrupted, the population of enterobacteria may increase (Rasmussen *et al.*, 1993). Transport stress and feed deprivation may contribute to this process. Events prior to slaughter can influence fermentative activity in the digestive tract and thus predispose animals to infection and faecal shedding (Rasmussen *et al.*, 1993). Kudva *et al.* (1997) found that when animals experienced a dietary change, there was a significantly large number of enterohaemorrhagic *E. coli* (EHEC) positive ewes at 4 days after the change-over. Thus dietary stress appears to contribute significantly to EHEC carriage. Rasmussen *et al.* (1993) demonstrated that dietary stress can result in replication of *E. coli* 0157:H7 in rumen fluid.

The manipulation of gastrointestinal flora through the methods described above could lead to the reduction of meat borne pathogens that enter the abattoir. This could lead to

a reduction in contamination of carcasses leading to increases in food safety and profitability. The welfare of animals could also be improved with decreased carcass weight losses and stress during transport to abattoirs, and overall improvement of animal health during their growth.

Chapter 3

Effect of preslaughter feeding system on weight loss, gut bacteria and the physico-chemical properties of digesta in cattle

3.1 ABSTRACT

The effects of four preslaughter feeding systems on gut microflora, digesta consistency, soiling of the hide with faeces, liveweight loss, dehydration, meat stickiness and plasma cortisol, were examined in 60 Angus steers. The feeding systems were: feeding hay for 48 or 24 hours before transport to slaughter, transporting cattle directly off pasture, and fasting for 24 hours before transport. Cattle were held overnight at the processing plant after 2 hours transport. At slaughter, pasture-fed cattle were carrying approximately 75 billion aerobic bacteria and 86 billion facultative anaerobes in their gastro-intestinal tracts. The number and type of bacteria were strongly influenced by the preslaughter feeding system. Rumen contents of the fasted animals were less acidic and more moist. Fasted animals had more *E. coli*, total coliforms and total anaerobes throughout the gut. The 48h hay-fed steers had higher numbers of *Enterococci* and fewer *E. coli*. The pasture groups had runnier faeces, and were dirtier after holding overnight at the processing plant. Treatments did not affect dehydration, but urinary sodium was negatively correlated with meat stickiness, suggesting some individuals were sufficiently dehydrated to influence this meat quality feature. It was concluded that feeding cattle hay for 48 hours before dispatch for slaughter provided several advantages over other preslaughter feeding systems.

3.2 INTRODUCTION

The New Zealand meat industry is based on ruminant-animals with proportionately larger digestive tracts than monogastric animals. Animals that have been well fed on pasture, without a fasting period before transport to slaughter, cause problems during transport and processing due to the composition and large volumes of digesta and faeces. These animals generally have increased faecal material on their hides compared to animals that have been fasted, which is a risk to meat hygiene (Figure 3.1). In the case of cattle, a very full digestive tract can be difficult to handle in the meat plant. Furthermore, the volumes of effluent on trucks, and discharge of stockyard waste and digesta into plant effluent streams, contributes greatly to a meat plant's effluent and pollutant loading and costs. To reduce these problems, farmers are generally encouraged to fast their cattle for a period before transport. Fasting decreases rumen and intestine weight (Bass and Duganzich, 1980) and fasted animals produce less faecal waste on stock trucks. However, fasting cattle for periods of about 20 hours or more can cause dehydration and carcass weight loss (Jacobson *et al.*, 1997). Fasting also decreases the dry matter content and particle size of digesta, which increases the risk to meat hygiene.

Serious human illnesses associated with *E. coli* O157:H7 including bloody diarrhoea, have been linked either directly or indirectly to cattle (Hancock *et al.*, 1997). Previous work has found that *E. coli* and salmonella grow rapidly during starvation (Brownlie and Grau, 1967). A period of 2-3 days starvation usually led to infection in the intestines of cattle, whereas cattle fed lucerne hay rapidly eliminated salmonella from the rumen (Brownlie and Grau, 1967). In rumen fluid of cattle, salmonella was found to increase 300 fold after 48 hrs starvation, whereas *E. coli* increased 2000 fold after 24 hours starvation (Brownlie and Grau, 1967).

Recently Diez-Gonzalez *et al.* (1998) found that acid resistant *E. coli* in the colon of animals fed grain before slaughter was 10^6 – fold greater than for hay-fed animals. Hovde *et al.* (1999) disagreed and suggested that feeding cattle hay may increase human infections with *E. coli* O157:H7.

Increasing fibre length and the proportion of fibrous feeds in the diet of stock preslaughter may stimulate water intake, buffering the animals against excessive dehydration. Fibrous feeds such as hay typically have a slower rate of passage through the animal when compared to fresh grass. Consequently the timely feeding of hay preslaughter may decrease the amount of faeces voided on the truck and in the stockyards at the plant, and increase the cleanliness of the animals. The timely plugging of the rumen with hay, along with partial emptying of the hindgut before transport to the abattoir, should decrease the amount of digesta entering the meatworks.

This study investigated the effects of preslaughter feeding systems on the microflora and consistency of the digesta and faeces. If the gut of the animal is over-full at the time the animals are sent for slaughter, and if the faeces are unduly runny, there is likely to be an increased risk of faecal contamination of the hide during transport. Runny faeces pose a particular hazard for carcass contamination with enteric bacteria because they are more difficult to control than firm faeces, and they are prone to spreading further.

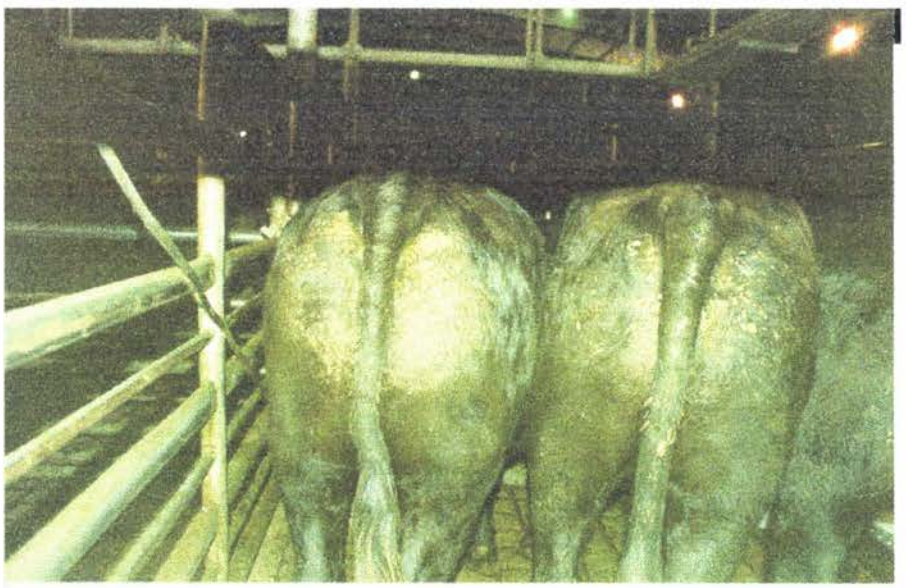

Figure 3.1 Faecal contamination of the hide.

The study reported here compared transport directly off pasture with either feeding hay for up to two days before transport, or fasting for 24 hours before transport.

Comparisons were made in terms of liveweight loss, differences in gut microflora, amount and consistency of the digesta and faeces, the extent of soiling of the hide with faeces, and levels of animal dehydration and gut fill.

3.3 MATERIALS AND METHODS

3.3.1 Animals and their treatments

Sixty Angus steers were purchased from a farm in southwest Waikato as three groups of 20. The cattle were transferred to paddocks at Ruakura and slaughtered about two weeks later at the Ruakura processing plant in February (summer), April (autumn) and June (winter). Prior to transfer to the Ruakura farm, they were not familiar with being handled. During the holding period at Ruakura, they were kept on pasture and consumed approximately 13 kg DM animal^{-1} day^{-1}. The dry matter intake was measured by taking quadrats of pasture before and after grazing. During the 2-week holding period the animals were weighed and faecal samples were taken directly from the rectum for determining the presence or absence of *Salmonella*. Faecal samples were collected from the rectum of each steer using a new glove for each animal. Samples were kept on ice and transported to the lab where analysis commenced 1.5 hrs after sampling.

All of the cattle were weighed two days before slaughter, and allocated to four weight-matched treatments with a total of 15 animals per treatment. The treatments were as follows. Five animals in each replicate were penned in a yard and fed hay for 48 hours before transport to the processing plant (48h hay; Figure 3.2). Five animals per replicate were fed pasture for one day and then hay for the final 24 hours before transport and slaughter (24h hay). Five animals per replicate were fasted for the last day before transport to slaughter (Fasted), and the remaining five animals in each replicate were fed pasture up to the time of transport to the processing plant (Pasture). The average stocking density of cattle when penned in yards was 40 kg m^{-2}.

Figure 3.2 Steer from 48h hay treatment group after 24 hours in pen.

The range in composition of the pasture and hay used during the preslaughter feeding period is shown in Table 3.1. The hay consisted largely of *Lolium perenne*, but included *Trifolium repens*, *Trifolium pratense*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Setaria glauca*, *Paspalum dilatatum*, *Plantago major*, *Plantago lanceolata*, *Polygonum aviculare*, *Taraxacum officinale*, *Crepis capillaris*, *Lapsana communis*, *Chenopodium album*, *Cerastium fontanum*, moss and species of *Poa*, *Agrostis*, *Rumex and Juncus*.

Table 3.1 Composition of the feeds used during the preslaughter feeding period.

Pasture	**February**	**April**	**June**
Dry matter, g $100g^{-1}$	29.7	21.7	13.6
Crude protein, g $100g^{-1}$ dry matter	12.4	15.8	11.8
Acid detergent fibre, g $100g^{-1}$ dry matter	30.0	28.4	28.1
Soluble sugars and starch, g $100g^{-1}$ dry matter	10.6	10.7	18.7
Estimated ME, MJ kg^{-1} dry matter	9.3	8.8	8.4

Hay	**February**	**April**	**June**
Dry matter, g $100g^{-1}$	85.4	85.2	81.7
Crude protein, g $100g^{-1}$ dry matter	7.5	6.1	8.7
Acid detergent fibre, g $100g^{-1}$ dry matter	38.0	39.2	35.7
Soluble sugars and starch, g $100g^{-1}$ dry matter	7.2	6.9	7.4
Estimated ME, MJ kg^{-1} dry matter	7.6	<8.0	8.1

3.3.2 Preslaughter sample collection

At the start of the treatment period (48hrs prior to transport), pre-transport and post-transport each steer was faecal-sampled using a new glove for each animal. The samples were stored in airtight pottles in a 1°C chiller for later analysis of faecal consistency and total solids. The steers were weighed at the start of the treatment period, and immediately before and after transport. The animals were penned in their respective treatments within the truck. The allocation of truck and trailer pens was randomised according to treatment over the three killing periods, and the allocation of the cattle to the respective treatments was arranged such that the stocking density in the truck and trailer pens was reasonably balanced across the treatments (mean stocking density on the truck and trailer was 376 kg m^{-2}). Transport lasted two hours.

The cattle were held overnight in separate pens at the processing plant, according to treatment (Figure 3.3). The average stocking density in the pens was 269 kg m^{-2} and it did not differ between treatments by more than 12 kg m^{-2}. The cattle were not fed during this period but had free access to water. Liveweight loss was recorded. During the final weighing before slaughter, they were scored for surface soiling with fresh faeces at five points on their body (bung, brisket, under neck, feet just proximal to the hoof and rump) as well as an overall score. A subjective 4-point scale was used, 0=none, 1=mild, 2=medium and 3=severe.

Figure 3.3 Cattle from the fasted treatment group in pen at abattoir.

3.3.3 Postslaughter sample collection

The cattle were slaughtered at approximately 16 hours after they left the farm. They were electrically stunned (head-to-brisket), and blood was collected in pottles containing EDTA, from the sticking wound for estimation of plasma protein and cortisol. The alimentary tract with its contents plus the bladder with its urethra were collected at evisceration. Urine was sampled from the bladder for estimation of sodium and creatinine concentrations. The rumen with reticulum, omasum plus abomasum, small intestine, caecum and large intestine were tied off to ensure that there was no mixing between these regions or loss of digesta. The gastrointestinal tract was divided into two sections with the rumen/reticulum, omasum and abomasum kept together and the other part of the gastrointestinal tract kept together. Digesta samples, collected in sterile 25ml universal tubes with lids, were taken from the rumen, duodenum, distal ileum, caecum, and distal colon from each set of viscera within 1 hour of slaughter. These samples were kept on ice and transferred directly to the microbiology laboratory for estimation of bacteria numbers.

Samples were collected from the same five sites along the gastrointestinal tract as for microbiological analysis to determine faecal consistency and total solids. They were collected in plastic 250ml plastic cups with lids and pH was measured within 5 minutes, before being stored in a 1°C chiller for later analysis. All samples were taken out of the chiller 24 hours before analysis to allow them to warm to room temperature.

Each gut region was weighed separately before and after emptying its digesta. The paunch waste was collected by cutting each paunch open and tipping the contents out into a bin in a similar manner to the commercial paunch "dry dumping" system. It was estimated that this method recovered approximately 90% of the total contents from each paunch. The method used for removing the digesta from the abomasum, omasum and intestines was somewhat different to the usual commercial method. Commercially, a gut-cutter with a washer are used. In the present study the omasal and abomasal contents were collected by hand and knife scraping, and the intestine contents were collected by manually squeezing them out of a cut end whilst winding the intestines

onto a windlass. The weight of gut contents retrieved by this method was inevitably an underestimate in comparison with the commercial method.

At 24 hours post mortem, pH and stickiness were measured as outlined below in the *longissimus dorsi* muscle between ribs 12 and 13.

3.3.4 Analytical methods

3.3.4.1 Microbiological methods

The microbiological measurements were conducted on samples of digesta from the rumen, duodenum, ileum, caecum and rectum from three of the four treatment groups only; 48h hay, pasture-fed, and fasted. Samples from the 24h hay treatment group were analysed in the June replicate only. The samples were pulverised with a Colworth stomacher before assaying for aerobic plate counts, and the number of *E. coli*, total coliforms, *Enterococci* and total anaerobes per gram of digesta. The term "total anaerobe" as used here means bacteria which develop on anaerobically incubated plates without precautions being taken during sample preparation, dilution and spreading to preclude contact with oxygen.

Salmonella in the faeces and digesta were assessed qualitatively using a selective enrichment procedure with Rappaport-Vassiliadis soya peptone broth followed by plating onto 50:50 brilliant green modified agar:xylose lysine desoxycholate agar (Reid and Cook, 1991).

Briefly the procedure was as follows. Approximately 25 grams of faeces was diluted (1:10 w/v) in buffered peptone water and mixed in a stomacher bag to prevent sample-induced pH changes, nutrient composition or other characteristics of the medium that may affect recovery of injured bacteria. Samples were incubated for 16-20 hours at 37°C.

After incubation, 0.1 ml of peptone water culture was added to 10ml of Rappaport-Vassiliadis soya peptone (RVS) broth (pre-warmed to 42°C). The samples were incubated for a further 24 hours at 42°C $\pm$ 0.5°C in an oven. This temperature is critical

for maximal recovery of salmonellae (Reid and Cook, 1991). A loopful (10µl) of the RVS culture was streaked onto a plate consisting of half Brilliant Green Modified Agar (BGM), and half, Xylose Lysine Desoxycholate Agar (XLD) to obtain single, well isolated colonies (Reid and Cook, 1991). The plates were then incubated for 18-24hrs at 37°C, at which time they were examined for salmonella colonies and presumptive positive *Salmonella* colonies were confirmed using a Latex Slide Agglutination Test (Serobact Salmonella Latex Slide Agglutination Test, Medvet Science Pty Ltd).

E. coli and total coliforms were assayed using AOAC Official Method 991 (1998). Briefly the procedure was as follows. Sample homogenates in dilution fluid were prepared using 10g of each sample in 90ml of dilution fluid. The samples were then stomached for two minutes. A dilution series was made using 4.5ml dilution tubes.

The top film of the Petrifilm™ *E. coli* plate (3M, U.S.A.) was raised and 1ml of sample was dispensed onto the centre of the bottom film. The top film was slowly rolled down onto the sample to prevent entrapment of bubbles. The sample was then distributed evenly within the circular well using gentle pressure. After 1 minute, the gel solidified and each plate was inoculated.

The Petrifilm™ *E. coli* plates were then incubated (clear side up) in stacks of 20 or less, at 37°C for 24 to 48 hours. Petrifilm™ *E. coli* plates contain Violet Red Bile (VRB) nutrients which are an indicator of glucuronidase activity and an indicator that facilitates colony enumeration. Most *E. coli* (97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony (*E. coli* O157:H7 is glucurondiase negative). Gas produced by lactose fermenting coliforms and *E. coli* is trapped by the top film as indicated by the blue to red-blue colonies seen on the plate. The blue colonies were then counted and the bacterial count per gram calculated.

Enterococci were determined by the method of Hartman *et al.* (1992). Sample homogenates in dilution fluid (0.1% peptone and 0.85% NaCl) were prepared and samples stomached for 2 minutes. Dilutions from 10^1 to 10^4 (0.1 ml) were spread onto divided Kenner Feacal (KF) streptococcus agar plates and incubated for 48 hours at 37°C. *Enterococci* appear as red or pink colonies on the agar plates. All pink and red colonies on the KF plates were counted and the count called "total *Enterococci*".

Although this agar is known to recover two non-enterococcus species (*Streptococcus bovis* and *Streptococcus equinus*) and is therefore more correctly a test for faecal streptococci than *Enterococci*, within the meat industry this test is referred to as the *Enterococcus* test.

Aerobic plate count and total anaerobe colony count were determined by the method of Swanson *et al.* (1992). Sample homogenates in dilution fluid (0.1% peptone and 0.85% NaCl) were stomached for 2 minutes. For aerobic plate counts, 0.1 ml of dilutions from 10^1 to 10^6 were spread onto divided Plate Count Agar (PCA) plates and incubated for 48 hours at 30° C. All colonies present were counted to give aerobic plate count. Total anaerobe colony counts were determined by counting all colonies present on divided Schaedler agar plates (Difco Laboratories, Becton Dickson and Company, U.S.A.) after 0.1ml of dilutions from 10^1 to 10^5 were spread onto the plates and incubated for 48 hours at 30° C.

The total burden of a particular bacterium in a gut region was calculated from the counts of that bacterium per gram of digesta multiplied by the weight of digesta in that region.

3.3.4.2 Physico-chemical properties of the digesta

The total solids samples were dried in an oven set at 80°C for 24 hours to estimate dry matter content.

Runniness of the faeces or digesta was measured using a splatter test. This test was based on the method of Ireland-Perry and Stallings (1993) that involved dropping faeces from dairy cows from a consistent height of approximately one metre and visually scoring the result. The scale developed was a subjective 4-point scale ranging from runny to dry. In the present trial 10 ml of material was expelled as a single bolus from a height of 1 m onto a sheet of paper (Figure 3.4). This height was used to imitate the height of the steer's rectum. The area covered by the material was measured by planimetry.

Figure 3.4 The standard 10ml syringe (with end removed: 15mm opening) used for expelling bolus onto brown paper and an example of resulting splat.

The physical phases of the digesta or faeces were assessed using a press test. A weighed amount (approximately 0.5 g ± 0.1g) of material was compressed between two perspex sheets with a device that applied a constant pressure (Figure 3.5). The lower plate was lined with a filter paper, and the upper plate was lined with kitchen cling film. After one minute compression the filter paper (Whatman® No.1, Qualitative) was removed, dried and the area of the separate phases measured by planimetry. In this test, the digesta or faeces separated into three distinct phases or rings (Figure 3.6). The innermost ring consisted of coarse fibrous matter (fibre ring). The next ring surrounding the fibre ring consisted of a dark opaque fluid (sludge ring), and the outermost ring was an almost translucent watery ring (free-water ring). Areas were expressed as $mm^2\ g^{-1}$ of sample.

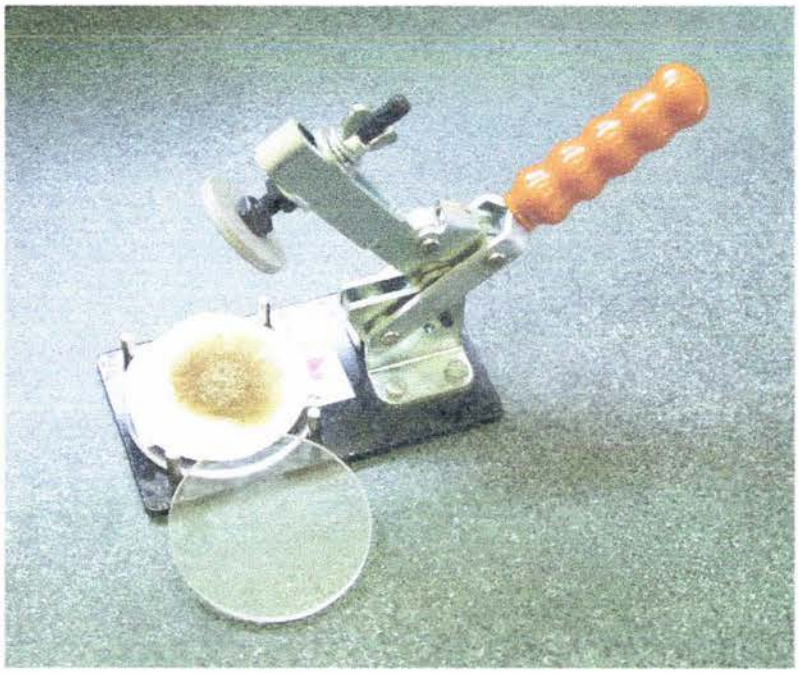

Figure 3.5 Device used in compression of faeces and digesta

Figure 3.6 Two examples of the three distinct rings obtained after press test (rumen sample on the left and rectal sample on the right)

3.3.4.3 Plasma cortisol, urinary sodium and creatinine

Plasma cortisol was measured by radio-immunoassay (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA), urinary sodium with a Nova Crt4 ion

selective electrode, and urinary creatinine using the Roche Boehringer Mannheim test (Cat. No.1489291 for the Hitachi 717 autoanalyser at 30°C).

3.3.4.4 Meat stickiness

Meat stickiness was measured on the *longissimus dorsi* in the region of the twelfth rib using a modification of the method described by Adam *et al.* (1986). It was based on measuring the force (grams) required to separate manually a plastic plate (14cm x 4cm) from the freshly cut surface of the meat after it had been pressed onto the surface firmly.

3.3.5 Statistical analysis

Statistical analyses were performed using linear regression, analysis of variance (ANOVA) and Chi-square using SigmaStat version 2 (Jandel Scientific Software, U.S.A).

All variables except *E. coli* counts and urine and plasma parameters (see below) were analysed by a one-way ANOVA (model: $y_{ij} = \mu + t_i + e_{ij}$; where t_i is the effect of the i^{th} treatment), with 3 treatments (Fasted, 48h hay, and Pasture). Replicate (February vs April vs June slaughter date) was originally included in the statistical model, but was not retained because it did have significant effects on any of the characteristics.

E. coli counts (per gram and total burden) were analysed using a Kruskal Wallis one-way ANOVA on ranks due to unequal variance of the treatments. The model used was: $H = 12/n(n + 1)\sum_i R^2_i/n_i - 3(n + 1)$ with n_i the number of observations (15) in the ith sample, $i = 3$, $n = \sum n_i$, and R_i is the sum of the ranks for the ith sample.

Urine creatinine, urine sodium, plasma protein and plasma cortisol were analysed using two-factor ANOVAs (model: $y_{ij} = \mu + t_i + u_j + tu_{ij} + e_{ij}$) with treatment and replicate as the design variables.

The Tukey Test was used for all pairwise comparisons of the mean responses to the different treatment groups. The Tukey Test controls the errors of all comparisons simultaneously.

Liveweight loss was analysed using ANOVAs with covariate adjustment for differences in pre-hold live weight. The data was analysed separately within each time point.

Linear regression and correlation analyses were used to evaluate the relationships between pairs of variables.

The prevalence of surface soiling of hides was analysed using a chi-square test to determine presence or absence of wet faeces. The model used was $\chi^2 = (n-1)s^2 / \sigma^2$; where s^2 is the sample variance which is an unbiased estimate the population variance, σ^2. The data was analysed as being either present or absent rather than as four levels according to the scoring system because of very low numbers in some categories.

3.4 RESULTS

3.3.1 Liveweight loss, dehydration and dressing-out percentage

The pasture and 48h hay fed animals lost more weight during transport than the other groups (Table 3.2). However, this difference did not apply to overall weight loss for the 16 hour period between leaving the farm and slaughter. There was a tendency for the 48h hay fed steers to lose less weight overall, but this was not significant ($P > 0.05$). The 48h hay fed group had a greater gut fill and a slightly lower dressing-out percent based on final live weight in comparison with the pasture-fed group. Otherwise there was little difference in the overall weight of gut contents between the groups. The pasture group had a significantly higher mean carcass weight (by 6-7 kg) than the 48h hay and fasted treatment groups after adjustments had been made for initial live weight.

The prevalence of surface soiling in the pasture-fed steers after overnight holding at the processing plant was 24% whereas in all the other treatments it was less than 7% ($P < 0.05$). The mean plasma protein and urine creatinine concentrations for the four treatments were in the normal range for cattle indicating that none of the groups were

dehydrated (Table 3.3). Meat stickiness as a measure of water content was, however correlated to urinary sodium (r = -0.408, $P < 0.01$), suggesting that animals which were actively retaining sodium in order to conserve body water may have been dehydrated to the extent that it affected the physical properties of their muscle. In addition, there was a negative correlation between urinary creatinine and the total weight of water in the alimentary tract kg^{-1} empty body weight (r = -0.421, $P < 0.01$). This implied that animals which had a low reserve of water left in their gut, were needing to concentrate their urine to conserve water.

Table 3.2 Effect of preslaughter feeding system on liveweight loss during the preslaughter period, and on killing out characteristics in cattle.
Means in a row without a common superscript letter were significantly different at $P < 0.05$. EBW = empty body weight. SED = standard error of difference. Carcass weight has been adjusted for the covariate live weight 48 hours before transport.

	48h Hay	24h Hay	Pasture	Fasted	SED
Number of steers	15	15	15	15	
Preslaughter period off-feed (hrs)	16	16	16	40	
Actual initial live weight (kg)	484	487	483	480	12
Actual final live weight (kg)	453	453	445	439	11
Live weight loss after commencement of preslaughter feeding period (g kg^{-1} EBW)	77	83	95	102	8
Live weight loss during transport (g kg^{-1} EBW)	22^{ab}	17^{bc}	23^{a}	12^{c}	2
Live-weight adjusted carcass weight (kg)	255^{a}	259^{ab}	261^{b}	254^{a}	3
Actual carcass weight (kg)	254	260	259	252	6
Gut contents at slaughter (kg digesta)	40.6^{a}	36.0^{ab}	32.8^{b}	34.5^{ab}	2.3
Gut contents at slaughter (g digesta kg^{-1} EBW)	102^{a}	88^{ab}	81^{b}	86^{ab}	5
Actual dressing-out % (based on initial live weight)	52.6	53.4	53.5	52.6	0.5
Actual dressing-out % (based on final live weight)	56.2	57.4	58.1	57.4	0.5

Table 3.3 Effect of preslaughter feeding system on measures of dehydration and stress in cattle.

Means in a row without a common superscript letter were significantly different at $P < 0.05$. EBW = empty body weight. SED = standard error of difference.

	48h Hay	24h Hay	Pasture	Fasted	SED
Number of steers	15	15	15	15	
Plasma protein ($g\ l^{-1}$)	75	79	79	80	1.7
Urine sodium ($mmol\ l^{-1}$)	30^{ab}	38^{ab}	44^{a}	10^{b}	9.0
Urine creatinine (mmol l^{-}	17	25	22	24	2.9
Total weight of water in gut contents (g water kg^{-1} EBW)	90^{a}	80^{ab}	74^{b}	82^{ab}	4
M longissimus thoracis stickiness (g)	268	239	244	263	19
M longissimus thoracis pH_{ult}	5.61	5.60	5.56	5.58	0.02
Plasma cortisol ($nmol\ l^{-1}$)	131	141	114	144	10.4

3.4.2 Microbiology

The effect of three of the preslaughter feeding systems (excluding the 24h hay treatment group) on the counts of *E. coli* g^{-1} of digesta in the five gut regions are shown in Figure 3.7. The corresponding total burden of *E. coli* in the rumen, small intestine, caecum and large intestine are shown in Figure 3.8. The fasted group had significantly higher concentrations and total burden of *E. coli* than the pasture-fed and the 48h hay-fed animals (Table 3.4; $P < 0.001$). The total burden of *E. coli* in the large intestine and the counts g^{-1} of *E. coli* in the rectum were lowest in the 48h hay treatment. The difference in rectal *E. coli* counts g^{-1} between the fasted and the 48h hay fed treatments, at about 10^3, was substantial.

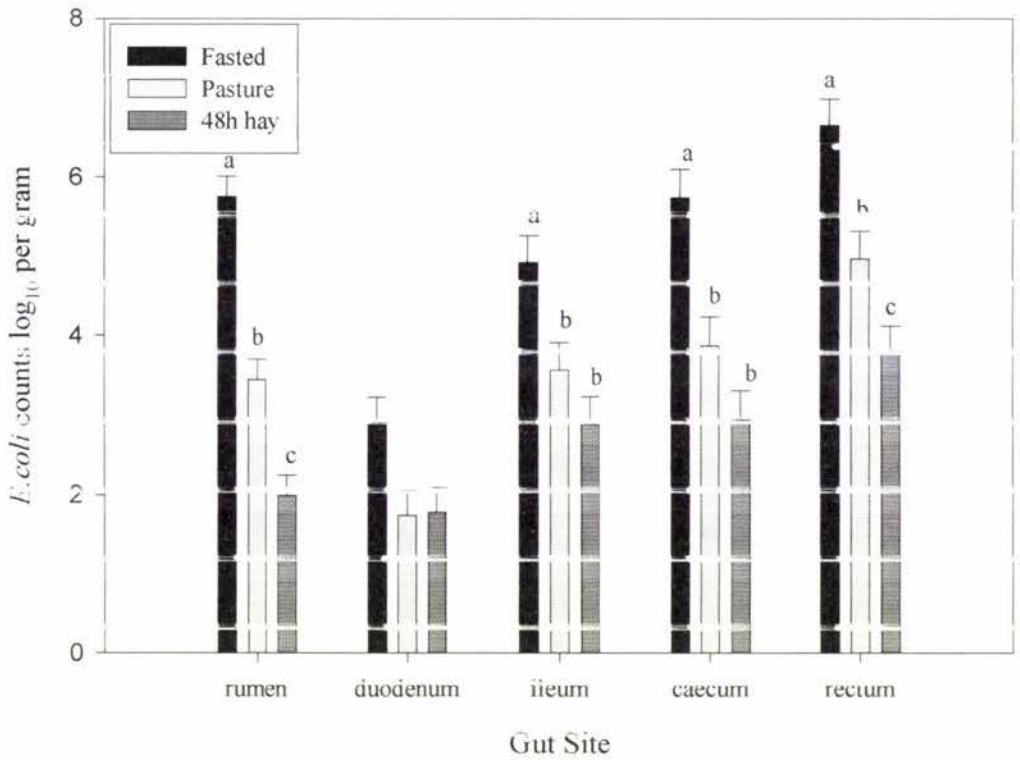

Figure 3.7 Effect of preslaughter feeding system on the counts of *E. coli* g^{-1} in the digesta at different sites in the gut.
Means within gut sites without a common letter were significantly different at P<0.05. Vertical bars are standard errors of means.

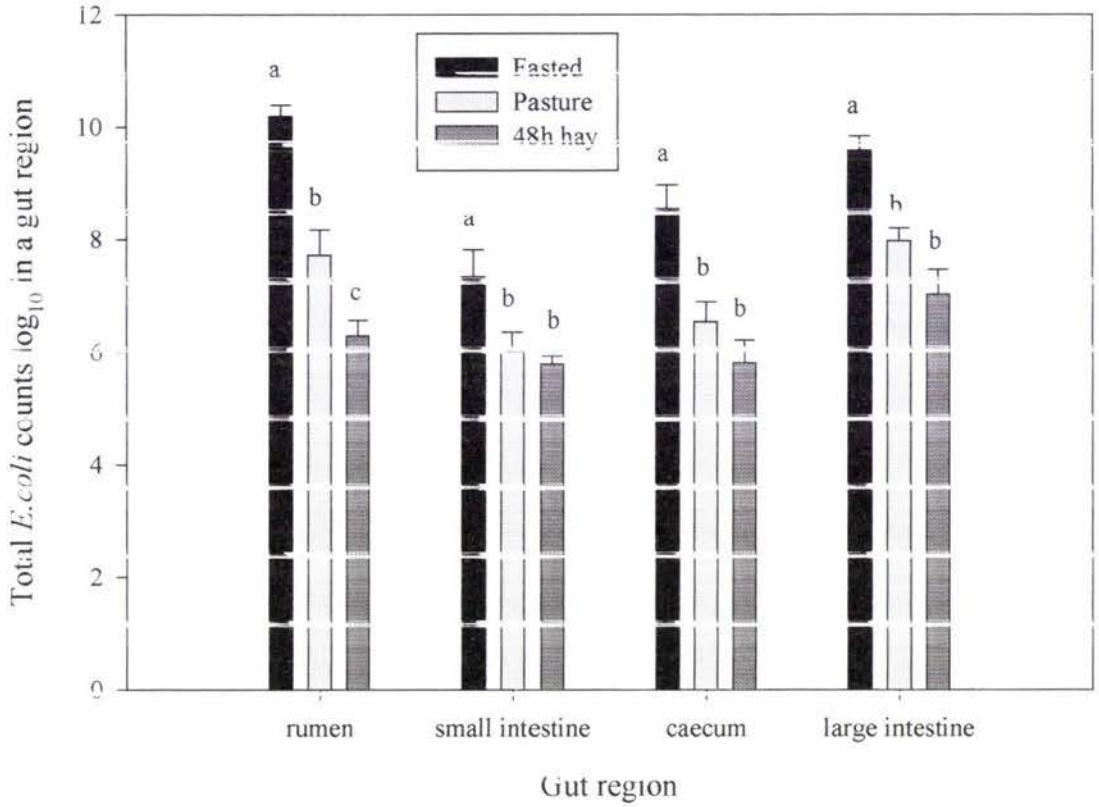

Figure 3.8 Effect of preslaughter feeding system on the total burden of *E. coli* in the different regions in the gut.
Means within gut sites without a common letter were significantly different at P<0.05. Vertical bars are standard errors of means.

The counts g^{-1} of *E. coli* were closely related to the aerobic plate count (APC g^{-1}). These two were positively correlated in all of the gut regions (range in correlation coefficients 0.65 to 0.76, $P < 0.001$) except the rumen, where they were negatively correlated ($r = -0.30$; $P < 0.05$). The preslaughter treatment differences for the APC g^{-1} were similar to those for the *E. coli* counts g^{-1} with higher levels for the fasted group (Table 3.4).

The fasted animals had higher counts g^{-1} and a higher total burden of total coliforms in the rumen and large intestine in comparison with the pasture and 48h hay fed animals ($P < 0.05$, Table 3.4). The fasted animals also had more total coliforms in the whole of the alimentary tract (Table 3.4).

The number of total anaerobes g^{-1} in rectal contents was higher in the fasted animals in comparison with the pasture and 48h hay-fed steers. The total burden of total anaerobes

in the entire alimentary tract was higher in the fasted steers in comparison with the pasture-fed animals ($P < 0.05$). The total burden of *Enterococci* was greatest in the 48h hay-fed steers but it was not significantly different from the other treatment groups (Table 3.4).

Table 3.4 Effect of preslaughter feeding system on bacteria in the faeces and the entire alimentary tract in cattle.
No SEDs are given for *E. coli* numbers as non-parametric Kruskal Wallis one-way ANOVA on ranks was used for analysis due to unequal variance of the treatments. Means in a row without a common superscript letter were significantly different at $P < 0.05$.

	48h Hay	Pasture	Fasted	SED
Counts (log_{10}) g^{-1} of faeces				
Aerobic plate count	6.109[a]	6.274[a]	7.358[b]	0.173
E. coli	3.716[a]	5.002[b]	6.650[c]	-
Total coliforms	4.634[a]	5.163[a]	6.968[b]	0.385
Enterococci	5.001	4.600	4.382	0.348
Total anaerobes	6.016[a]	6.410[a]	7.439[b]	0.226
Total burden in the alimentary tract, log_{10}				
Aerobic plate count	11.889[a]	10.875[b]	10.255[b]	0.111
E. coli	7.609[a]	8.779[a]	10.480[b]	-
Total coliforms	8.142[a]	9.161[a]	10.700[b]	0.288
Enterococci	9.901	9.383	8.982	0.329
Total anaerobes	11.867[a]	10.932[b]	11.189[b]	0.157

Five out of the 60 steers were *Salmonella* positive.

In general, the differences between the treatments in faecal *E. coli* counts were present along the whole length of the gut except for the duodenum (Figure 3.7). Within the rumen, it appeared that moist conditions and a relatively high pH favoured the growth and survival of *E. coli*, as counts of *E. coli* in the rumen digesta were negatively correlated with rumen digesta dry matter percent, and were positively correlated with rumen contents pH (Table 3.5). Total coliforms counts and total burden in the rumen were also higher at a relatively high rumen pH. In the rumen, *Enterococci* and *E. coli*

were negatively correlated, and total anaerobes were positively correlated to the number of aerobes (Table 3.5).

Table 3.5 Correlation coefficients for the linear regression relationships between pH, dry matter percent and the log number of bacteria in the rumen contents ($y = a + bx$) across three treatments (n = 45).
APC = aerobic plate count.

y	*x*	*r*	*P* <
E. coli g^{-1}	Dry matter %	-0.751	0.001
E. coli g^{-1}	pH	0.517	0.001
Total *E. coli*	pH	0.518	0.001
Total coliforms g^{-1}	pH	0.554	0.001
Total total coliforms	pH	0.543	0.001
E. coli g^{-1}	*Enterococci* g^{-1}	-0.466	0.001
Total *E. coli*	Total *Enterococci*	-0.484	0.001
Anaerobes g^{-1}	APC g^{-1}	0.775	0.001
Total anaerobes	Total aerobes	0.810	0.001

3.4.3 Physico-chemical properties of the digesta

The dry matter content of the digesta in the fasted animals was lower than that in the other preslaughter feeding treatments (Figure 3.9). The rumen contents, in particular, were wetter and they were exceptionally runny in the fasted animals (Tables 3.6 and 3.7). However, their large intestine must have been more active in taking up this extra water, as their faeces had a similar dry matter percentage to that of the other animals (Figure 3.9).

Table 3.6 Effect of preslaughter feeding systems on characteristics of rumen contents in cattle.
Means in a row without a common superscript letter were significantly different at $P < 0.05$.

	48h Hay	24h Hay	Pasture	Fasted	SED
Number of steers	15	15	15	15	
Rumen contents pH	7.013 [a]	7.100 [a]	7.033 [a]	7.380 [b]	0.056
Rumen contents dry matter %	11.089 [a]	9.643 [ab]	9.095 [b]	5.485 [c]	0.469
Rumen *E. coli* $\log_{10}$ g^{-1}	1.988 [a]	-	3.439 [b]	5.752 [c]	0.337
Rectum *E. coli* $\log_{10}$ g^{-1}	3.783 [a]	-	4.969 [b]	6.650 [c]	0.354

Table 3.7 Effect of preslaughter feeding system on faeces and digesta consistency.
Means in a row without a common superscript letter were significantly different at $P < 0.05$.

	48h hay	24h hay	Pasture	Fasted	SED
Size of the sludge phase (mm^2 g^{-1})					
Rectal contents – before transport	2365 [ac]	2312 [ac]	3597 [b]	2230 [c]	209
Rectal contents – after transport	2448 [ab]	2560 [a]	3239 [b]	2497 [a]	207
Rumen contents – at slaughter	1983 [a]	2283 [a]	3040 [bc]	2939 [c]	167
Caecal contents – at slaughter	2606 [a]	2895 [ad]	3943 [bc]	3379 [cd]	177
Runniness (mm^2 $10ml^{-1}$)					
Rectal contents – before transport	2268 [a]	2124 [a]	4567 [b]	1590 [a]	478
Rectal contents – after transport	2908 [abc]	2559 [a]	5124 [b]	2383 [c]	607
Rumen contents – at slaughter	3329 [a]	5754 [a]	5860 [a]	11402 [b]	718
Caecal contents – at slaughter	9959 [a]	10729 [a]	10290 [a]	18881 [b]	2238

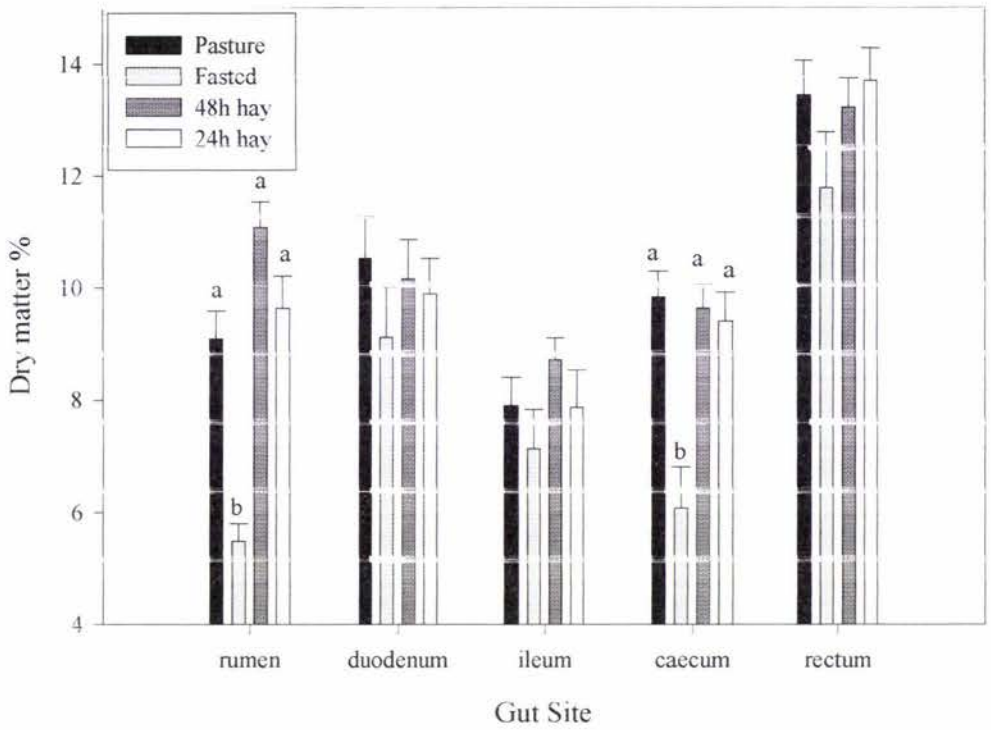

Figure 3.9 Effect of preslaughter feeding system on the dry matter content of the digesta at different sites in the gut.

Means within gut sites without a common letter were significantly different at $P<0.05$. Vertical bars are standard errors of means.

Rectal contents, which were runny as measured by the splatter test, had a low dry matter and a relatively large sludge phase (Table 3.8). This association was particularly noticeable for the pasture-fed steers which had a larger sludge phase and runnier rectal contents (Table 3.7). In many respects, the preslaughter feeding treatment differences in the sludge phase of the rectal contents were also present for the digesta in the caecum and the rumen, which suggests that the preslaughter feeding treatment influenced this feature throughout the digestive tract (Figure 3.10). Whereas, this did not apply to runniness. The fasted group had the runniest rumen and caecal contents, but the pasture group had the runniest rectal contents.

Table 3.8 Correlation coefficients for the linear regression relationships between the physico-chemical properties of the faeces sampled from cattle before transport ($y = a + bx$) across all treatments (n=60).

Y	*x*	*r*	*P* <
Runniness	Area of the sludge phase	0.86	0.001
Runniness	Area of free-water phase	-0.49	0.001
Runniness	Dry matter % of faeces	-0.82	0.001
Dry matter %	Area of the sludge phase	-0.75	0.001

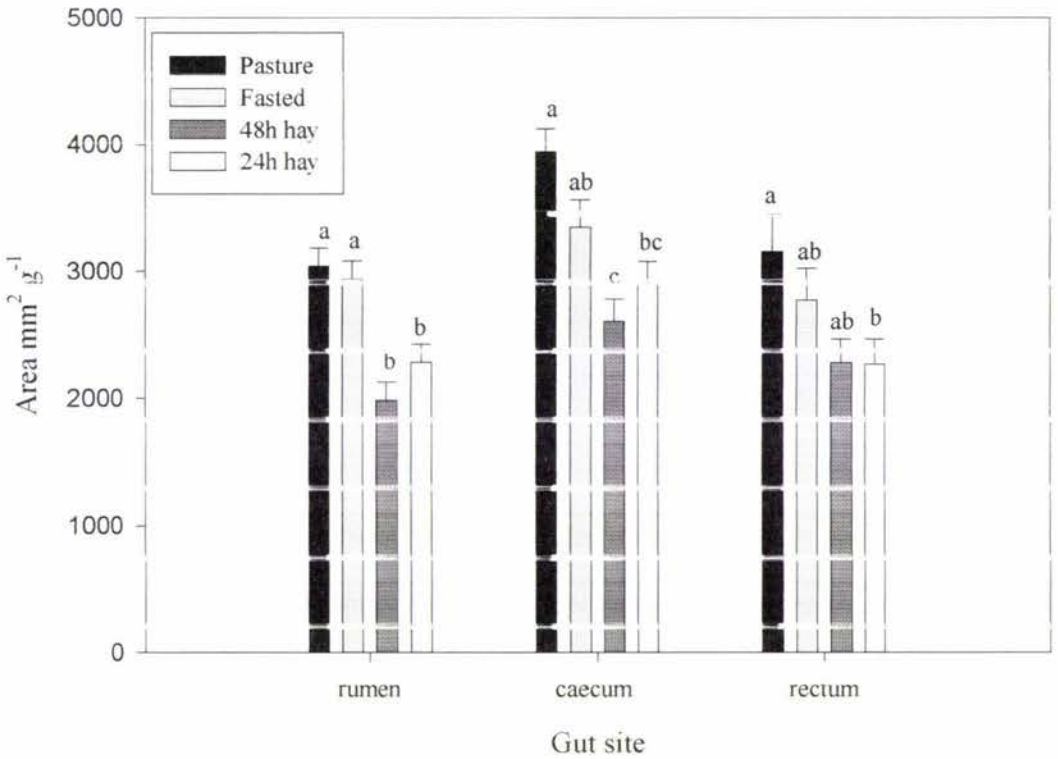

Figure 3.10 Effect of preslaughter feeding system on the size of the sludge phase of the digesta at different sites in the gut.
Means within gut sites without a common letter were significantly different at P<0.05. Vertical bars are standard errors of means.

3.5 DISCUSSION

The New Zealand meat processing industry is putting greater effort into ensuring that meat is *E. coli*-free. There have been two reasons for the move towards zero tolerance for this group of bacteria. Firstly, the presence of *E. coli* in foods of animal origin indicates that there is a moderate to high risk that the food has been contaminated at some stage with faeces, non-potable water or diluted faecal matter (Eley, 1996). Whether the *E. coli* that are detected could give rise to gastro-intestinal problems in consumers, depends on the type and number of *E. coli* that are present (Eley, 1996). Secondly, certain types of *E. coli*, and in particular *E. coli* 0157:H7, have emerged as serious pathogens in children and convalescent subjects, and it has been decided that one way of ensuring that this particular organism is not likely to be present in a food, is to control or exclude all *E. coli*, using a zero tolerance policy. Live cattle have been implicated as the main reservoir for foodborne *E. coli* 0157:H7, and the main sites where they proliferate and survive are the rumen, reticulum and omasum (Brown *et al.*, 1997, Chapman *et al.*, 1997).

E. coli contaminate beef carcasses in the processing plant either directly, from spillage of digesta and faeces onto the carcass during processing through faecal matter that is transferred from the hide, or by contact with staff or equipment that have been contaminated. Over the past thirty years there has been an increasing focus on minimising the transfer of bacteria by these routes, and, in general, the hygiene standards now used in processing carcasses for export are high in comparison with other nations (Cook *et al.*, 1997). However, insufficient effort has gone into reducing the burden of undesirable bacteria that are carried by the live animal into the processing plant. One of the aims of this study was to examine the total burden of *E. coli* and other enteric bacteria that are commonly present in cattle in New Zealand when they are slaughtered, and to consider some simple strategies for reducing this burden.

It is well-recognised that the counts of *E. coli* in freshly voided faeces at the time cattle are presented for slaughter can be highly variable (Davidson and Taylor 1978). For example, in some animals there is less than 10^2 counts g^{-1} faeces whereas others can have more than 10^7 g^{-1}. Part of the reason for this sizeable variation could be differences in time off feed. Brownlie and Grau (1967) showed that fasting cattle before slaughter

encourages the growth of *E. coli* in the rumen, and this could lead to more *E. coli* in the faeces (Jordan and McEwen 1998). The present study supported that finding and showed that it also applied to the total burden of *E. coli* within the whole alimentary tract (Table 3.4). In addition, it was found that the counts of *E. coli* in the faeces were related to the counts in the rumen contents ($r = 0.68$, $P<0.001$). An important implication is that differences in *E. coli* counts between animals in the rumen will probably persist further down the tract. This in turn implies that manipulating the growth and survival of *E. coli* in the rumen before transport could be an effective way of influencing the counts of *E. coli* in the faeces during the critical preslaughter period.

Several factors could be influencing the numbers of *E. coli* in the rumen. There are a number of theories that may help explain the suppression of *E. coli* in the 48-hour hay group in the present trial. These include the effect of coumarins, the 'broom' effect where bacteria attach to increased dietary fibre and are swept out of the gastrointestinal tract, the effect of antimicrobials produced by fungi on hay, the introduction of hay-borne bacteria acting as an inoculant of competitive bacteria and the alteration of dietary substrates affecting the population dynamics to the advantage of *E. coli* competitors.

In this study *E. coli* numbers in the rumen contents were related to rumen contents pH and dry matter content. A low dry matter content with a near-neutral pH favoured *E. coli*. Fasting the animals before transport favoured the formation of a higher pH-lower dry matter rumen environment relative to the other treatments. The pattern of treatment differences persisted along the gut after the digesta had passed from the rumen and through the duodenum. This could indicate that the entry rate of *E. coli* from the rumen into the small intestine was an important factor influencing subsequent faecal *E. coli* numbers.

In practical terms, the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay. The reduction in faecal *E. coli*, in comparison with the fasted animals, was substantial; approximately 10^3 g^{-1}. However, hay feeding did not have much effect on rumen contents pH and dry matter, and so it is suspected that some other factors mediated the *E. coli*-suppressing effect provided by the hay. These could include the presence of inhibitory compounds that may have been present in the hay, but to a lesser extent in the pasture. Duncan *et al.* (1998) observed that the coumarins,

esculetin, umbelliferone and scopoletin, which are normal constituents in some swards, inhibited the growth of *E. coli* 0157 *in vitro*. These components were not monitored in the present study, but the hay that was used in this study did include species such as *Anthoxanthum odoratum, Trifolium repens, Trifolium pratense, Polygonum aviculare* and *Taraxacum officinale* which normally contain some of those compounds (Murray *et al.*, 1982). An alternative explanation is that the lower counts of *E. coli* in the hay-fed animals could have been due to greater competition from other bacteria. The hay-fed animals had more *Enterococci* in their alimentary tracts, and there was a negative relationship between *Enterococci* and *E. coli* in the rumen (Table 3.4 and 3.5). Some *Enterococci* are known to inhibit the growth of *E. coli*, *Salmonella*, *Shigella* and *Enterobacter* species, and have been used in other species as probiotics because of their beneficial effect on the gut microflora (Franz *et al.*, 1999).

During the trial only five cattle tested positive to *Salmonella*. The positives were all found in the 2-week holding period before the start of the treatments. Even though the cattle were sampled every 2 days to overcome the problem of intermittent shedding there were not high numbers of positive samples.

When cattle are fasted before transport, the weight of gut contents decreases but the material usually becomes more liquid (Bass and Duganzich 1980). In other words the dry matter % of the digesta decreases with progressive starvation. The present study confirmed this effect and produced the following new information. Firstly, if cattle are taken directly from pasture and transported to the processing plant where they are slaughtered 16 hours later, the total weight of gut contents and the total weight of water in the gut contents are similar to those seen in cattle which are fasted for 24 hours before transport. Secondly, even though cattle that are fasted usually have more moist digesta, the cattle that were pasture-fed up to the time of trucking had more runny faeces. The faeces from the pasture-fed cattle contained a higher proportion of the sludge phase, relative to the fibre and free-water phases. In addition, the pasture-fed animals were dirtier, in terms of fresh faecal soiling on the hide. A previous study in bulls showed that if cattle are transported whilst in the fed state, they are more prone to getting stressed (Jacobson and Cook 1997), and it is expected that this may be a contributory cause of an increased rate of emptying of runny hind gut material in the truck although not reflected in different cortisol levels. In summary, feeding pasture on

the farm up to the time of transport has only a small effect on the composition of gut contents but it has an adverse affect on faeces consistency and stock cleanliness.

Preslaughter liveweight loss was comparable in the four treatments, and it is suspected that the differences in weight loss during transport were a reflection of differences in gut fill at the start of the journey. The dressing-out percentages for each treatment group increased when based on the final weight taken just before slaughter. The differences in dressing-out percent are probably due to gut fill in the three treatments that were fed during the preslaughter period. Dehydration was not a marked feature in any of the treatments, but the animals appeared stressed in their behaviour.

3.6 CONCLUSION

In conclusion, the preslaughter feeding treatment which offered the most advantages was providing hay for 48 hours before despatch. This method helped to reduce the gut burden and excretion of *E. coli* and it helped to keep the animals clean. Cattle that were transported directly from pasture had runny faeces and ended up with more surface soiling on the hide. Fasted animals produced less effluent during transport, but they had high levels of *E. coli* in their rumens and faeces at slaughter. The way the cattle were fed before slaughter had little effect on the amount of weight they lost.

Chapter 4

Effect of feeding pasture-raised cattle different conserved forages on *Escherichia coli* in the rumen and faeces

4.1 ABSTRACT

The effects of eight different pre-slaughter diets on gut microflora, pH and dry matter concentration were evaluated in 112 slaughter-weight, pasture-raised heifers. Hide dirtiness at slaughter, plasma cortisol, meat pH and stickiness, and liveweight loss, were also examined. The diets for 48 hours before transport were: meadow hay, lucerne hay, red clover hay, perennial ryegrass hay, haylage, haylage supplemented with maize silage, pasture, or 24 hours pasture followed by 24 hours fasting. Slaughter was 16 hours after the start of 2 hours transport. Fasted animals had the highest counts of rumen *E. coli* and total coliforms compared to other treatments ($P<0.001$), while red clover hay and haylage had the lowest ($P<0.05$). Faecal *E. coli* counts were also highest for fasted animals ($P<0.05$). Rumen *E. coli* and total coliforms counts were positively correlated with rumen pH ($P<0.001$), and negatively correlated with fibre intake ($P<0.001$). Diet significantly affected the pH of the rumen and faeces ($P<0.001$), with fasted animals having the highest rumen pH and haylage and red-clover hay-fed animals the lowest. Dry matter (%) of the rumen contents was lowest for the fasted group ($P<0.001$). The mean carcass weight of fed heifers was 5kg heavier than of the fasted heifers at the same initial weight ($P<0.05$). A pH/volatile fatty acid (VFA) dependent mechanism is suggested as the main mechanism for *E. coli* suppression by feeds used in the study. Coumarins may also have contributed to lower ruminal and faecal *E. coli* counts. Feeding cattle conserved forages before transport for slaughter may prove beneficial in reducing digesta levels of microbes, including potential pathogens, and in reducing carcass weight losses.

4.2 INTRODUCTION

The presence of *Escherichia coli* on meat or meat products indicates faecal or digesta contamination. It raises the possibility that the product could be carrying other bacteria of enteric origin, such as Salmonella, Yersinia, Campylobacter or pathogenic *E. coli* including the enterohaemorrhagic serotype O157:H7.

The recent focus of the meat industry, with respect to carcass contamination control, has been on dressing procedures and worker hygiene. However, the single greatest source of *E. coli* contamination in the meat plant environment – the animals themselves, remains relatively unchallenged. Contamination of the carcass can derive from the rumen via reflux though the oesophagus, and from accidental viscera puncture during dressing. Indirect contamination can result from the transfer of faecal material from the hide to the carcass. Much of this opportunity for carcass contamination may be unavoidable (Dickson and Anderson, 1992). However, it may be possible to reduce the food safety risk associated with digesta and faecal contamination by: (1) decreasing the counts of potential pathogens within the digestive tract of the animal so that if digestive tract contamination of the carcass does occur it carries a lower pathogen danger, or; (2) improving the hide cleanliness of stock presented for slaughter, and therefore reducing the risk of indirect carcass contamination.

Recent studies have indicated that a reduction in concentration of *E. coli* in the intestinal tract of cattle may be achieved by manipulation of the diet (Jordan and McEwan, 1998; Diez-Gonzalez *et al.*, 1998; Russell *et al.*, 2000), although there is controversy about this effect (Hancock *et al.*, 1999; Russell and Diez-Gonzalez, 1999; Duncan *et al.*, 2000), and opposing results with hay feeding have also been reported (Hovde *et al.*, 1999). In the majority of these studies, the original diet fed to the cattle was grain, and the dietary change investigated was a switch to 100% hay. In New Zealand, much of Europe, Australia and South America, cattle finishing systems are mostly based on pasture diets rather than grain. It is of interest, therefore, to determine whether or not acute dietary shifts from pasture have an effect on *E. coli* counts in both the rumen and faeces of pasture-finished animals at slaughter.

In the previous chapter, feeding meadow hay to pasture-finished cattle for 48 hours before transport to slaughter was shown to greatly reduce the concentration and total numbers of *E. coli* along the length of the digestive tract. Hay feeding or fasting before transport also reduced the amount of faecal soiling on the hide at presentation for slaughter compared to that on animals transported straight from pasture. These results indicated that both direct and indirect carcass contamination risks could be reduced by pre-slaughter dietary shifts.

The present study was undertaken to further investigate this effect, using a broad range of common New Zealand conserved feeds to determine their action against *E. coli* in the digestive system of pasture-fed cattle destined for slaughter. Specific feeds were selected with a view to identifying the anti-microbial features present in, or produced by, the different feeds. As meat production parameters and animal welfare are important features in a commercial slaughter setting, the diet shifts were also evaluated for effects on meat stickiness and ultimate pH, carcass weight and the stress indicator hormone, cortisol.

4.3 METHODS

4.3.1 Experimental Design and Animals

One hundred and twelve rising two-year old crossbred beef heifers from Hawkes Bay and Opotiki were brought to the Ruakura Dairy Research Corporation Farm Number 4 in two lots: 54 heifers in March 2000, and 58 heifers in May 2000. They were grazed at Ruakura for about two weeks before slaughter and were weighed again two days before slaughter at which time 14 animals were allocated to each of eight, weight-matched treatments.

There were six feeding treatments for the 48 hours before transport for slaughter: meadow hay, lucerne hay, red clover hay, perennial ryegrass hay, haylage, and haylage supplemented with maize silage. The remaining two treatments were: transport straight from pasture, and; pasture followed by 24 hours fasting before transport. Feed levels of hays, haylages and pasture were calculated to provide an estimated metabolisable energy intake sufficient for maintenance and growth at 750 g d^{-1} for heifers of that age

and weight (Joyce *et al.*, 1975; Figures 4.1 and 4.2). Intakes of cattle in the 48 hours pasture-fed and 24 hours pasture followed by 24 hours fasting treatments were estimated by visually assessing the pasture cover before and after grazing (as kg dry matter per hectare), then calculating the herbage consumption for the given area of paddock. A trained professional, who regularly estimates pasture cover and employs self-calibration checks against actual pasture dry matter using cut quadrat techniques, made the visual assessments of pasture cover (Pers. Comm. K. MacDonald).

Figures 4.1 and 4.2 Heifers from the rye grass treatment group in their pens (2 or 3 heifers per pen).

The term "haylage" refers to pasture ensiled in a large plastic wrapped bale. In New Zealand, haylage pasture is typically cut when the pasture is long. It is wilted before baling and then wrapped in plastic without further chopping, using purpose-built machinery (White *et al.*, 1999). Haylage used in the present study was mixed sward and made as described above.

As maize silage was a novel feed for the animals, it was provided in the maize silage-supplemented haylage treatment at a maximum allowance of 2kg wet weight of maize silage per heifer per 24 hours, to reduce the risk of ruminal acidiosis occurring.

The ryegrass hay was made from the residue left after harvesting for seed, and consequently it was particularly stalky and fibrous. Five animals only were in the March ryegrass hay treatment and their intake of the hay was particularly poor. Consequently, in May when the remaining nine animals in the ryegrass treatment were slaughtered, the ryegrass hay was flavoured with molasses (5% molasses weight per kg dry matter) diluted in water and sprayed on the hay immediately before it was presented to the cattle.

The heifers were weighed immediately before and after 2 hours transport to an abattoir. Each treatment group was separately penned at the abattoir and held overnight. The cattle had no access to feed at this time, but water was freely available. Live-weight was recorded early the next morning, and animals scored subjectively for visual surface soiling with wet faeces. These "dirtiness scores" were assessed for each individual heifer while standing in the weigh-crate on the morning of slaughter. The presence of visible wet faeces on the hide was recorded as either present or absent for each of five regions on the animal: feet at joint, butt, around the anus, brisket, and under the neck, plus an overall score for the whole animal. Results are expressed as percentage (%) of treatment group that were faeces-free in each of the five assessed regions.

The cattle were slaughtered about 16 hours after leaving the farm. They were electrically stunned (head-to-brisket) and exsanguinated by thoracic sticking. Blood from the sticking wound was collected for analysis of plasma protein and cortisol. After evisceration the diaphragm was visually assessed for appearance of blood splash. The entire digestive system for each animal was collected about 25 minutes after slaughter,

and digesta samples taken immediately from the rumen and rectum (Figures 4.3 and 4.4). Digesta samples were assessed for pH, dry matter and microbiological characteristics.

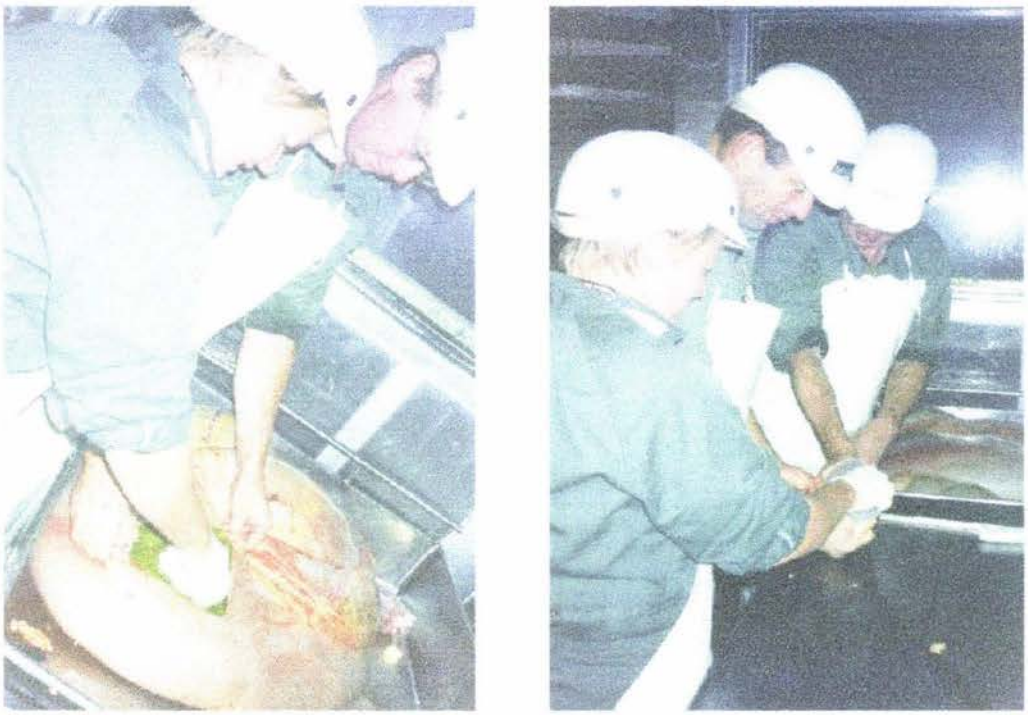

Figure 4.3 Collection of samples from the rumen (left) and rectum (right).

Figure 4.4 pH measurement of samples from the rumen and rectum.

Hot carcass weights were recorded for each individual animal. Twenty-four hours after slaughter, meat pH and stickiness were measured on the fresh-cut surface of the right *longissimus dorsi* at the position of the 13th rib.

4.3.2 Microbiological and analytical methods

Digesta and feed samples were homogenised with a Colworth stomacher before assaying for aerobic plate counts, and the number of *Escherichia coli*, total coliforms, total anaerobes, *Enterococcus faecalis* and generic *Enterococci* (in the May replicate of the trial only). In this report the term "total anaerobes" is applied to bacteria which developed on anaerobically incubated plates without precautions being taken to preclude oxygen contact during sample collection, preparation, dilution and plate spreading.

E. coli and total coliforms were assayed using AOAC Official Method 991 (1998). Briefly the procedure was as follows. Sample homogenates in dilution fluid were prepared using 10g of each sample in 90ml of dilution fluid. The samples were then stomached for two minutes. A dilution series was made using 4.5ml dilution tubes.

The top film of the Petrifilm^{TM} *E. coli* plate (3M, U.S.A.) was raised and 1ml of sample was dispensed onto the centre of the bottom film. The top film was slowly rolled down onto the sample to prevent entrapment of bubbles. The sample was then distributed evenly within the circular well using gentle pressure. After 1 minute, the gel solidified and each plate was inoculated.

The Petrifilm^{TM} *E. coli* plates were then incubated (clear side up) in stacks of 20 or less, at 37°C for 24 to 48 hours. Petrifilm™ *E. coli* plates contain Violet Red Bile (VRB) nutrients which are an indicator of glucuronidase activity and an indicator that facilitates colony enumeration. Most *E. coli* (97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony (*E. coli* O157:H7 is glucurondiase negative). Gas produced by lactose fermenting coliforms and *E. coli* is trapped by the top film as indicated by the blue to red-blue colonies seen on the plate. The blue colonies were then counted and the bacterial count per gram calculated.

Enterococci were determined by the method of Hartman *et al.* (1992). Sample homogenates in dilution fluid (0.1% peptone and 0.85% NaCl) were prepared and samples stomached for 2 minutes. Dilutions from 10^1 to 10^4 (0.1 ml) were spread onto divided Kenner Feacal (KF) streptococcus agar plates and incubated for 48 hours at 37°C. *Enterococci* appear as red or pink colonies on the agar plates. All pink and red colonies on the KF plates were counted and the count called "total *Enterococci*". *Enterococci faecalis* were indicated by red colonies with yellowing of the agar. Although this agar is known to recover two non-enterococcus species (*Streptococcus bovis* and *Streptococcus equinus*) and is therefore more correctly a test for fecal streptococci than *Enterococci*, within the meat industry this test is referred to as the *Enterococcus* test.

Aerobic plate count and total anaerobe colony count were determined by the method of Swanson *et al.* (1992). Sample homogenates in dilution fluid (0.1% peptone and 0.85% NaCl) were stomached for 2 minutes. For aerobic plate counts, 0.1 ml of dilutions from 10^1 to 10^6 were spread onto divided Plate Count Agar (PCA) plates and incubated for 48 hours at 30° C. All colonies present were counted to give the aerobic plate count. Total anaerobe colony counts were determined by counting all colonies present on divided Schaedler agar plates (Difco Laboratories, Becton Dickson and Company, U.S.A.) after 0.1ml of dilutions from 10^1 to 10^5 were spread onto the plates and incubated for 48 hours at 30° C.

Plasma cortisol was determined by radio-immunoassay (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA).

Meat pH was measured using a Mettler Toledo MP125 pH meter with a Mettler Toledo InLab 427 spear-tipped probe.

Meat stickiness was determined by measuring the force required to draw a standard area plastic plate from the fresh cut surface of the meat (method derived from Adam *et al.*, 1986). It was based on measuring the force (grams) required to separate manually a plastic plate (14cm x 4cm) from the freshly cut surface of the meat after it had been pressed onto the surface firmly.

Nutritional compositions of feeds were determined using near-infra red reflectance spectrometry prediction, calibrated with wet chemistry methods (FeedTech, AgResearch, Palmerston North).

The amount of coumarins ingested by cattle in the treatments were estimated from expected concentration of coumarins in the species and age of plants ingested (Murray *et al.*, 1982), and on the mean dry matter intake of that plant matter by each treatment in the 48 hours prior to transport. Expected coumarin ingestion by each treatment was then ranked from highest to lowest (5 to 0).

4.3.3 Statistical analysis

Statistical analyses were performed using linear regression and correlation, analysis of variance (ANOVA) and Chi-square using SigmaStat version 2 (Jandel Scientific Software, U.S.A).

Microbial data was analysed by a one-way ANOVA (model: $y_{ij} = \mu + t_i + e_{ij}$; where t_i is the effect of the i^{th} treatment), with 8 treatments. Replicate (March vs May slaughter date) was originally included in the statistical model, but was not retained because it did have significant effects on any of the characteristics except for *E. faecalis*.

E. faecalis counts in the rumen and rectum were analysed using one-factor ANOVA, analysing all cattle with replicate as the design variable.

As the ryegrass hay treatment differed between March and May replicates, data for the ryegrass treatment were first examined to determine if there were differences between the replicates that were inconsistent with those found between replicates of other treatments. When inconsistencies were noted, the two replicates of the ryegrass treatment were analysed as separate treatments.

E. coli counts were analysed using a Kruskal Wallis one-way ANOVA on ranks due to unequal variance of the treatments. The model used was:
$H = 12/n(n + 1)\sum_i R^2_i/n_i - 3(n + 1)$ with n_i the number of observations (15) in the ith sample, $i = 3$, $n = \sum n_i$, and R_i is the sum of the ranks for the ith sample.

The Tukey Test was used for all pairwise comparisons of the mean responses to the different treatment groups. The Tukey Test controls the errors of all comparisons simultaneously.

Liveweight and carcass weight data were adjusted for the co-variate "pre-treatment live-weight" and analysed using restricted maximum likelihood techniques (REML). The data was analysed separately within each time point.

Linear regression and correlation analyses were used to evaluate the relationships between pairs of variables.

The prevalence of surface soiling of hides was analysed using a chi-square test to determine presence or absence of wet faeces. The model used was $\chi^2 = (n-1)s^2 / \sigma^2$; where s^2 is the sample variance which is an unbiased estimate the population variance, σ^2. The data was analysed as being either present or absent rather than as four levels according to the scoring system because of very low numbers in some categories.

4.4 RESULTS

4.4.1 Live-weight, Animal Stress and Meat Quality

4.4.1.1 Live-weight and Carcass Weight

There were no significant treatment differences in mean adjusted live-weight after 48 hours of the feeding treatments on farm, or after transport, or lairage, or in adjusted carcass weights (Table 4.1). However, when analysed as fed (all treatments together except fasted and March ryegrass treated cattle) versus fasted, fed animals had significantly higher live weights after the pre-transport treatments ($P<0.01$), after

transport ($P<0.01$) and after lairage ($P<0.01$) (Table 4.1). The carcass weights of fed heifers was also significantly greater by about 5kgs than those which were fasted ($P<0.05$) (Table 4.2).

Table 4.1 Mean adjusted live-weights of heifers fed different pre-transport diets or fasted before 2hrs transport to an abattoir then overnight lairage.

SED = standard error of difference. Means were not significantly different ($P<0.05$).

Pre-transport dietary treatment	Number of Animals	Adjusted Start Weight (kg)	Pre-transport Weight (kg)	Post-transport weight (kg)	Post-lairage Weight (kg)
Meadow hay	14	402.4	404.9	393.3	386.5
Lucerne hay	14	402.4	401.7	389.1	383.3
Red clover hay	14	402.4	414.6	400.9	391.3
Ryegrass	14	402.4	400.8	394.2	384.0
Haylage	14	402.4	413.7	395.8	392.4
Maize +haylage[3]	14	402.4	408.2	391.8	386.2
Pasture	14	402.4	394.8	380.4	371.9
Fasted	14	402.4	376.9	369.9	365.0
SED		*2.57*	*7.76*	*7.25*	*7.17*

Table 4.2 Mean (adjusted for the co-variate "pre-treatment live-weight") carcass weights and dressing-out percentages based on pre-treatment liveweight of heifers fed or fasted before 2 hours transport and 16 hours lairage before slaughter.
SED = standard error of difference.

Pre-transport dietary treatment	Live weight adjusted carcass weight (kg)	Dressing-out percent
Meadow hay	210.7	51.79
Lucerne hay	214.0	52.81
Red clover hay	211.8	52.50
Ryegrass hay	207.3	51.80
Haylage	211.0	52.36
Maize + haylage	211.5	52.46
Pasture	212.5	53.05
Fasted	206.2	51.72
P	*<0.05*	*0.343*
SED	*4.1*	*0.46*

4.4.1.2 Plasma Protein and Cortisol

Haylage-fed animals had a significantly lower plasma protein concentration than fasted, pasture and meadow hay-fed animals ($P<0.01$; Table 4.3). Plasma cortisol at slaughter was not significantly influenced by pretransport dietary treatments or fasting.

Table 4.3 Mean plasma protein concentration of blood collected at slaughter of cattle subjected to different dietary treatments before transport and overnight lairage.
SED = standard error of difference. Means without common superscripts are significantly different ($P<0.05$).

Pre-transport dietary treatment	Plasma protein g l^{-1}
Meadow hay	76.2 [a]
Lucerne hay	74.1 [ab]
Red clover hay	73.1 [ab]
Ryegrass hay	74.9 [ab]
Haylage	66.9 [b]
Maize + haylage	72.9 [ab]
Pasture	79.1 [a]
Fasted	78.4 [a]
SED	*2.1*

4.4.1.3 Meat pH, Stickiness and Blood Splash

Meat pH was not significantly affected by the preslaughter dietary treatments or fasting. The mean pH of all carcasses was 5.6 (± 0.01). Four animals had a pH greater than 5.8, and the highest pH recorded was 6.03. The occurrence of the high pH animals did not appear to be related to any particular treatment. Meat stickiness was also not significantly affected by preslaughter dietary treatments. There was no blood splash present in any of the carcasses.

4.4.2 Feed characteristics and intakes

The nutritional and microbiological characteristics of the feeds used in the trial are shown in Tables 4.4 and 4.5, respectively. The quantity of dry matter consumed by cattle and coumarin content of feedstuffs in the different treatments is shown in Table 4.6.

Table 4.4 Nutritional composition of feeds.
(DM = dry matter (g/100g DM), Protein (g/100g DM), Lipid (g/100g DM), Ash (g/100g DM), ADF = acid detergent fibre (g/100g DM), NDF = neutral detergent fibre (g/100g DM), CHO = soluble carbohydrates (g/100g DM), OMD = organic matter digestibility (%), Lactic acid (g/100g DM), Ammonia (g/100g DM), ME = metabolisable energy (MJ/kg DM), - = not measured).

	Meadow hay	**Lucerne hay**	**Red clover hay**	**Ryegrass hay**	**Haylage**	**Maize + haylage[1]**	**Pasture (March)**	**Pasture (May)**
DM	81.3	82.4	79.1	83.6	42.7	43.0	30.1	15.0
Protein	11.6	16.2	13.4	4.0	12.2	11.6	14.2	28.3
Lipid	2.0	1.9	1.9	1.3	4.1	4.0	3.8	6.1
Ash	10.8	7.0	11.2	5.2	6.6	6.3	8.8	10.7
ADF	34.1	36.8	33.2	46.2	36.8	35.1	32.7	18.4
NDF	47.2	16.6	45.6	75.1	56.1	53.9	57.8	39.2
CHO	7.8	7.1	8.9	5.0	4.3	8.6	6.2	11.5
pH	-	-	-	-	4.3	4.2	-	-
OMD	59.7	56.7	65.7	50.0	65.3	-	55.8	82.2
Lactic acid	-	-	-	-	5.2	5.1	-	-
Ammonia	-	-	-	-	129.9	115.6	-	-
ME	8.7	8.4	9.5	7.5	10.5	10.5	8.0	11.6

[1] Maize + haylage = maize silage-supplemented haylage

Table 4.5 Microbiological composition of feeds, colony forming units (CFU) $\log_{10} g^{-1}$.
(Anaerobes = facultative anaerobic plate count, Aerobes = aerobic plate count, - = not measured).

	Meadow hay	**Lucerne hay**	**Red clover hay**	**Ryegrass hay**	**Haylage**	**Maize + haylage (March)[1]**	**Maize + haylage (May)[1]**	**Pasture (March)**	**Pasture (May)**
E. coli	0	0	0	1	0	0.43	0	0	0
Total coliforms	5.71	4.61	6.08	0	0	0.61	0.69	5.72	0
Total anaerobes	5.36	4.16	5.84	6.12	5.61	5.61	5.69	7.01	5.93
Aerobes	6.91	5.53	5.52	7.08	4.59	4.83	4.81	7.66	6.13
E. faecalis	0	0	2.29	1.18	4.63	4.52	4.27	2.85	0
Enterococci	-	-	-	-	-	-	0.19	-	2.60

[1] Maize + haylage = maize silage-supplemented haylage

Table 4.6 Dry matter intake of different treatments over the 48 hours before transport (total kg animal^{-1}) and expected coumarin content of feedstuffs.

	Meadow hay	**Lucerne hay**	**Red clover hay**	**Ryegrass hay (March)**	**Ryegrass hay (May)**	**Haylage**	**Maize + haylage[2]**	**Pasture (March)**	**Pasture (May)**	**Fasted (March)[3]**	**Fasted (May)[3]**
Dry matter intake (kg 48h^{-1})	9.6	11.9	14.2	4.2	8.9	13.2	8.2	10.7	14.1	4.7	7.4
Coumarin rank[1]	4	5	5	0	0	3	2.5	3	2.5	1.5	1.5

[1] "Coumarin rank" is the expected intake of coumarins ranked from 0-5, where 0 = no coumarins ingested and 5 = maximal coumarins ingested. The rank is constructed from the expected coumarin concentration of feedstuffs (from Murray *et al.*, 1982) and the quantity of the feedstuffs eaten in the 48 hours before transport.
[2] Maize + haylage = maize silage-supplemented haylage.
[3] Intakes for fasted animals refer to the first 24 hours of pasture grazing, as in the second 24 hours, feed intake of the fasted animals was zero.

4.4.2 Characteristics of digesta

The pH of the rumen contents at slaughter was significantly affected by pre-transport dietary treatments with the fasted animals having the highest rumen pH, and the haylage and red clover hay-fed animals the lowest ($P<0.001$) (Figure 4.5). In contrast, the pH of rectal contents for the red clover hay-fed animals was the highest of the treatments, while the faecal pH remained low for the haylage-fed animals (Figure 4.5).

Rumen pH (Figure 4.5) was significantly negatively correlated with both neutral detergent fibre (NDF) intake ($r = -0.40$; $P<0.001$; Table 4.4 and 4.6), and acid detergent fibre (ADF) intake ($r = -0.34$; $P<0.001$, Table 4.4 and 4.6). For example, the NDF intakes (kg $48h^{-1}$) of the haylage and fasted groups were 7.4 and 2.8 kg, respectively, and the corresponding mean rumen pH values were 6.93 and 7.48.

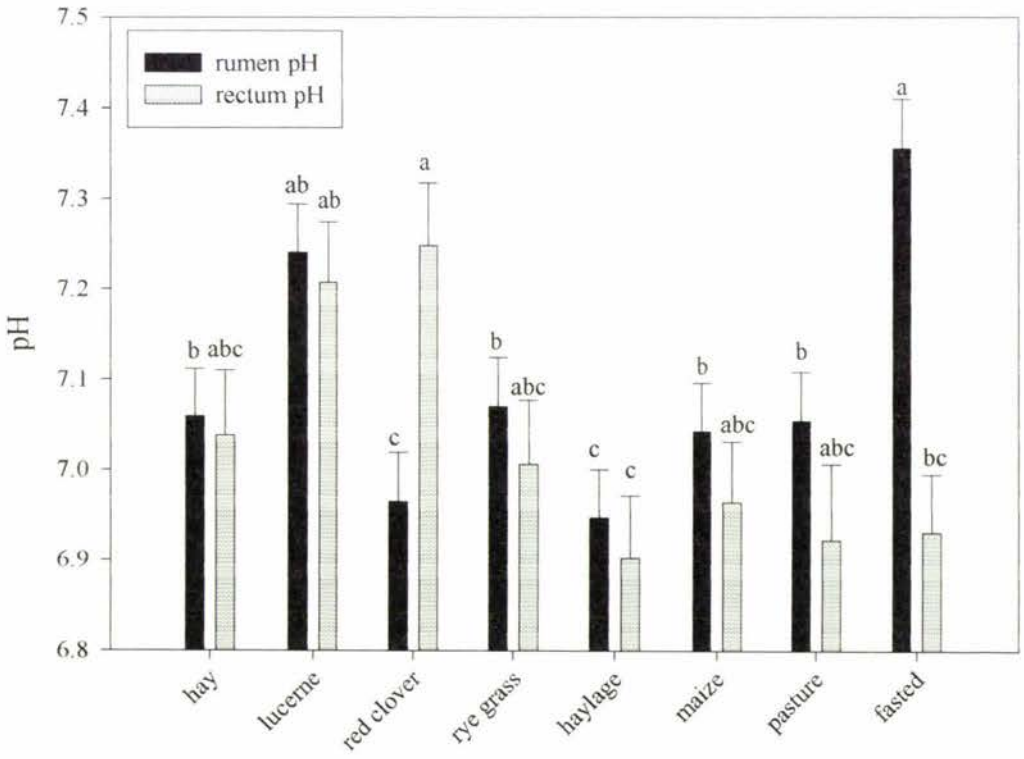

Figure 4.5 Effect of preslaughter diet on pH of rumen and rectum contents.
Means without common superscripts are significantly different ($P<0.05$).

The preslaughter dietary treatments had a highly significant effect on the dry matter percentage of the rumen contents at slaughter (P<0.001). Animals fed lucerne hay had drier rumen contents than haylage fed animals, while the dry matter percentage of fasted animals rumen contents was significantly lower than that of all other treatments (Figures 4.6 and 4.7). Overall, rumen dry matter percentage was higher by an average of about 0.7% in cattle slaughtered in the May trial replicate, than in March (P<0.01).

The dry matter percentage of faeces collected from the rectum after slaughter (overall mean 12.3% ± 0.29 sem) was not affected by the preslaughter dietary treatments or by the seasonal separation of the two trial replicates.

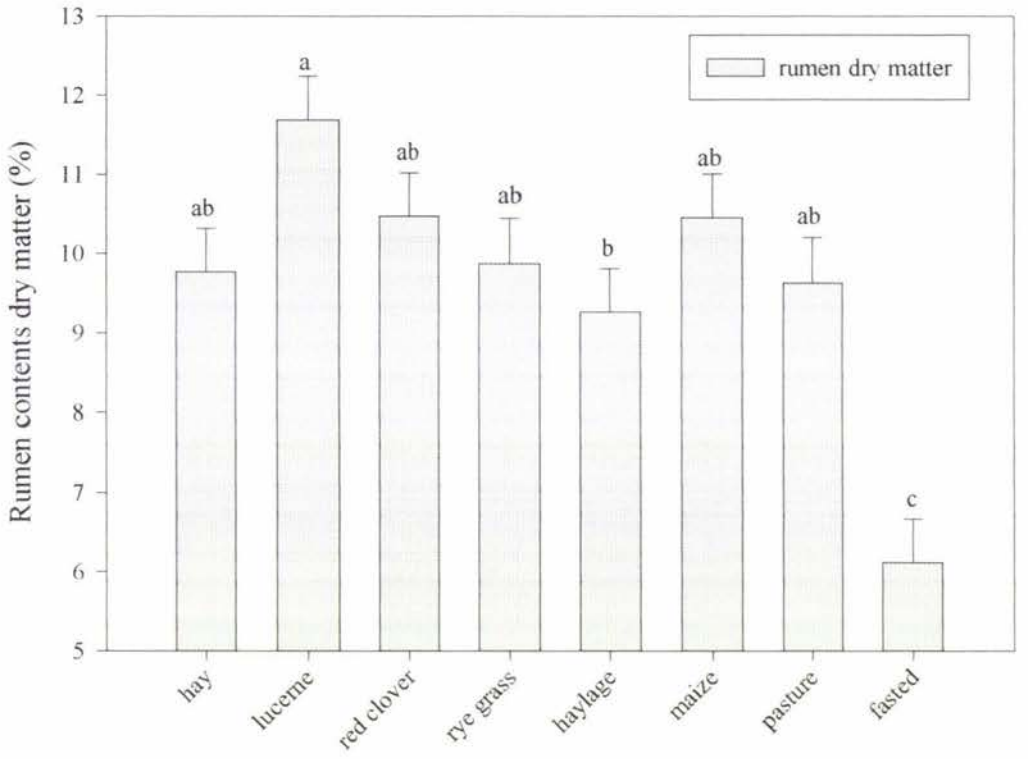

Figure 4.6 Effect of preslaughter diet on the dry matter percentage of rumen contents. Means without a common superscript are significantly different (P<0.05).

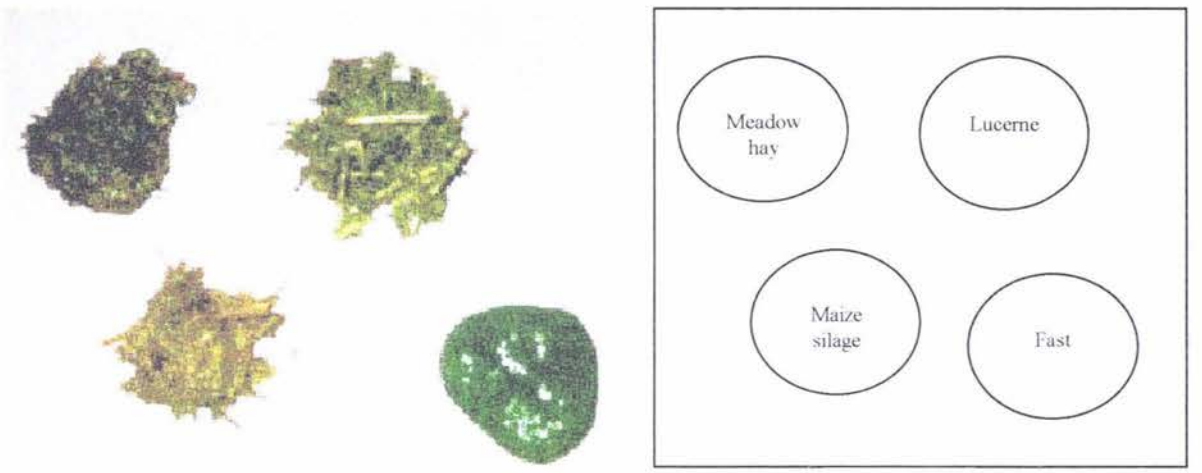

Figure 4.7 Visual differences in rumen contents of cattle feed different diets preslaughter.

4.4.3 Microbiology

4.4.3.1. E. coli

The *E. coli* counts in rumen contents for the eight pretransport feeding treatments show that the fasted animals had substantially higher counts of *E. coli* than all the feed treatments ($P<0.001$) (Figure 4.8), with the difference between mean fasted animal rumen *E. coli* counts and the next closest treatment (ryegrass hay) being 2.0 $\log_{10}$ *E. coli* g^{-1}. The treatment with the lowest *E. coli* counts g^{-1} (red clover hay) was 4.6 $\log_{10}$ counts g^{-1} lower than that of the fasted animals (Table 4.7).

Rectal counts of *E. coli* were also greatest in the fasted animals ($P<0.05$), although there were no significant differences between other treatments (Figure 4.8).

Table 4.7 Effect of pre-transport feeding regime on bacteria (counts of colony forming units $\log_{10}$ g^{-1}) in post-slaughter rumen and faeces of cattle.

(Anaerobes = facultative anaerobic plate count, Aerobes = aerobic plate count; Means without common superscripts are significantly different (P<0.05) within microbial group and site of digesta sample; SED = standard error of difference)

	E. coli	Total coliforms	Total anaerobes	Aerobes	*E. faecalis*	*Enterococci*[1]
Rumen contents						
Meadow hay	2.22 [bc]	2.45 [bc]	6.00 [a]	5.85 [a]	1.33	5.99 [a]
Lucerne hay	2.24 [bc]	2.39 [bc]	4.54 [bc]	4.44 [c]	1.24	3.89 [bc]
Red clover hay	0.78 [c]	1.88 [c]	5.32 [ab]	5.01 [b]	0.76	4.26 [b]
Ryegrass	3.39 [b]	3.64 [b]	5.16 [b]	5.17 [b]	0.72	4.77 [b]
Haylage	1.61 [c]	2.02 [c]	4.24 [c]	4.37 [c]	0.63	4.15 [b]
Maize +haylage[3]	1.53 [c]	1.95 [c]	3.95 [c]	4.44 [c]	0.46	2.76 [c]
Pasture	3.02 [b]	3.76 [b]	5.17 [b]	5.22 [b]	1.12	4.71 [b]
Fasted	5.37 [a]	5.54 [a]	5.31 [ab]	5.43 [ab]	1.01	4.56 [b]
SED	*0.40*	*0.34*	*0.19*	*0.13*	*0.34*	*0.24*
Rectum contents						
Meadow hay	5.33	5.36 [ab]	5.03 [ab]	5.23 [abc]	1.56	4.76 [a]
Lucerne hay	4.91	4.77 [b]	4.95 [ab]	5.06 [c]	1.22	3.28 [b]
Red clover hay	4.69	4.85 [ab]	5.33 [ab]	5.33 [abc]	1.64	4.88 [a]
Ryegrass	5.03[2]	6.54 [a]	5.56 [a]	5.92 [ab]	1.10	4.49 [ab]
Haylage	5.38	5.53 [ab]	4.94 [ab]	5.18 [bc]	1.33	3.68 [ab]
Maize + haylage[3]	4.73	4.92 [ab]	4.65 [b]	5.06 [c]	1.40	4.10 [ab]
Pasture	5.74	5.71 [ab]	5.37 [ab]	5.52 [abc]	2.28	3.97 [ab]
Fasted	6.05 [4]	6.20 [ab]	5.55 [a]	5.91 [a]	1.72	4.20 [ab]
SED	*0.61*	*0.39*	*0.20*	*0.17*	*0.32*	*0.27*

[1] Enterococci means are for May replicate only

[2] Mean is for May replicate only

[3] Maize + haylage = maize silage-supplemented haylage

[4] When blocked for statistical analysis as "fed" versus "fasted", the rectum contents of fasted animals were significantly higher in *E. coli* than those given feed (P<0.05), although there were no statistical differences in rectum *E. coli* counts between the different feeds.

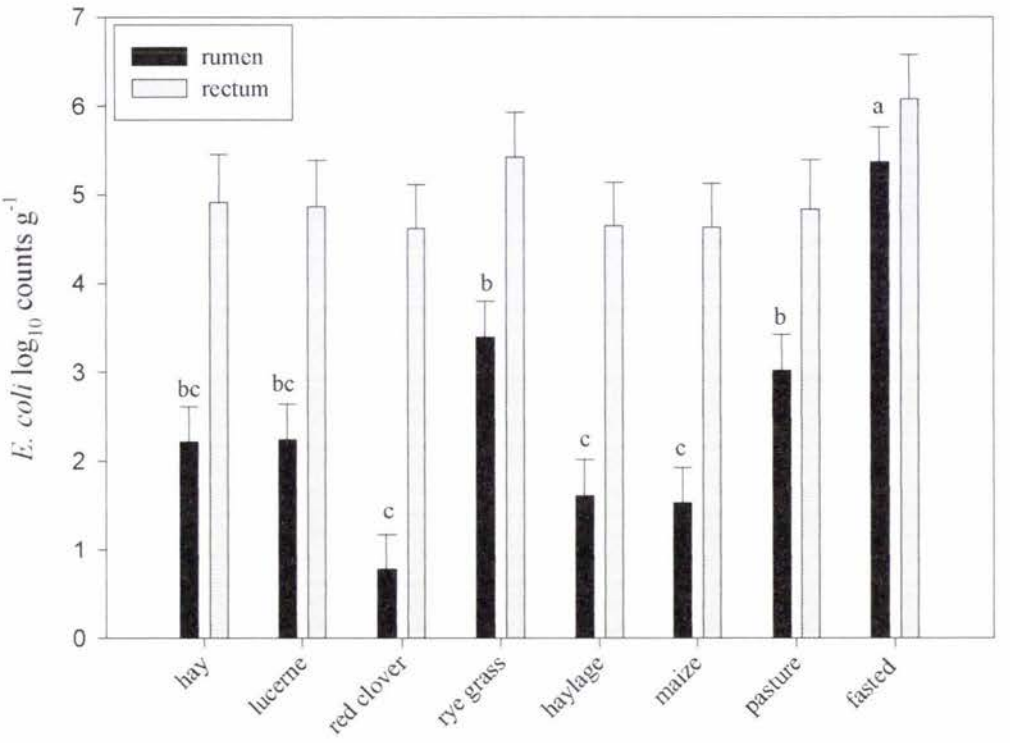

Figure 4.8 Effect of preslaughter diet on *E. coli* (log_{10} counts g^{-1}) in rumen and rectum contents.
Means without a common superscript are significantly different ($P<0.05$).

E. coli in the rumen were positively correlated with pH ($r = 0.33$, $P<0.001$, Figures 4.5 and 4.8), and negatively correlated with dry matter percentage ($r=-0.44$, $p<0.001$, Figures 4.6 and 4.8). Rumen *E. coli* counts were also closely related to rumen total coliforms, aerobic plate count and total anaerobic plate count (Table 4.8). Rumen *E. coli* and total coliform counts were significantly negatively correlated with ADF and NDF intakes ($P<0.001$; Table 4.8). Rumen and rectum *E. coli* counts were significantly negatively correlated with expected coumarin intake rank, before and after exclusion of red clover hay from the analysis (Table 4.8).

Table 4.8 Significant correlation coefficients between microbial $\log_{10}$ counts and physico-chemical characteristics of rumen and rectum contents based on data for all cattle.
*** = P<0.001; ** = P<0.01; NS = not significant.

		r	
y	***x***	**Rumen**	**Rectum**
E. coli g^{-1}	pH	0.33 ***	NS
E. coli g^{-1}	Dry matter %	-0.44 ***	NS
E. coli g^{-1}	ADF intake	-0.59 ***	NS
E. coli g^{-1}	NDF intake	-0.48 ***	NS
E. coli g^{-1}	Coumarin intake[1]	-0.40 ***	-0.32 **
E. coli g^{-1}	Coumarin intake[2]	-0.31 **	-0.31 **
Total coliforms	pH	0.35 ***	NS
Total coliforms	Dry matter %	-0.45 ***	NS
Total coliforms	ADF intake	-0.59 ***	NS
Total coliforms	NDF intake	-0.46 ***	NS
Total coliforms	Coumarin intake[1]	-0.40 ***	-0.39 ***
Total coliforms	Coumarin intake[2]	-0.35 ***	-0.38 ***
E. coli g^{-1}	Total coliforms	0.96 ***	0.95 ***
E. coli g^{-1}	Aerobes	NS	0.63 ***
E. coli g^{-1}	Total anaerobes	NS	0.52 ***
Total coliforms	Aerobes	0.30 ***	0.66 ***
Total coliforms	Total anaerobes	NS	0.56 ***
Total anaerobes	Aerobes	0.84 ***	0.84 ***

[1]Expected coumarin intake rank (constructed from Murray *et al.*, 1982)
[2]Expected coumarin intake rank (constructed from Murray *et al.*, 1982) with red clover hay excluded from statistical analysis
Coumarin rank is the expected intake of coumarins ranked from 0-5, where 0 = no coumarins ingested and 5 = maximal coumarin intake. The rank is constructed from the expected coumarin concentration of feedstuffs (from Murray et al., 1982) and the quantity of the feedstuffs eaten in the 48 hours before transport.

4.4.3.2 Total Coliforms

The counts of total coliforms in the rumen were affected by the preslaughter dietary treatments in a similar way to that of *E. coli*, in that fasted animals had a higher counts than all other treatments, and haylage, maize silage-supplemented haylage and red clover hay-fed animals had the lowest counts (Table 4.7). Rumen total coliform counts were significantly negatively correlated with ADF and NDF intakes (P<0.001; Table 4.8).

Total coliforms in the rectal contents of slaughtered animals were significantly higher ($P<0.05$) in the faeces of animals fed ryegrass hay than lucerne hay (Table 4.7). While these treatments differed significantly from each other, they did not differ significantly from the other treatments.

Both rumen and rectum contents total coliform counts were significantly negatively correlated with expected coumarin rank, before and after exclusion of red clover hay from the analysis.

4.4.3.3 Enterococci and E. faecalis

The counts of generic *Enterococci* (Table 4.7) in the rumen were significantly higher in animals fed meadow hay than in any other treatment ($P<0.001$). Maize silage-supplemented haylage-fed animals had the lowest number of *Enterococci* in their rumens. Rumen *Enterococci* counts for other treatments were similar, and intermediate between the meadow hay and maize silage-supplemented haylage silage treatments (Table 4.7).

Treatment differences in rectal *Enterococci* counts were less distinct than in the rumen, with lucerne hay-fed animals shedding significantly ($P<0.05$) less Enterococci than meadow or red clover hay-fed cattle (Table 4.7).

Despite the influence of pretransport diets on generic *Enterococci*, they had little impact on the counts of *E. faecalis*, a component of generic *Enterococci*, in either the rumen or rectum contents of the cattle. *E. faecalis* numbers represent approximately a quarter that of generic *Enterococci*, indicating that species of *Enterococci* other than *E. faecalis* were predominant in both the rumen and rectum contents. *E. faecalis* numbers were higher in both the rectum and rumen in samples collected in May, in comparison to March samples (Table 4.9).

4.5 DISCUSSION

Previous studies have indicated that fasting animals prior to slaughter can increase ruminal and faecal *E. coli* counts. Brownlie and Grau (1967) and Grau *et al.* (1969) found fasting increased *E. coli* in the rumen of sheep. Jordan and McEwen (1998) determined that fasting for 48 hours increased *E. coli* in the faeces of cattle by 1.4-2.4 log counts g^{-1}, depending on the preceding diet (feedlot ration versus high-roughage ration). A previous study conducted at this institute reported that fasting animals for 42 hours (including transport and overnight lairage) before for slaughter, substantially increased both the concentration and total numbers of *E. coli* within the digestive system, in comparison with animals fed before transport (Gregory *et al.*, 2000). The present trial also demonstrated that fasting for 40 hours (including transportation and overnight lairage) before slaughter increased *E. coli* in the rumen and rectum at slaughter, while the feeding of some conserved forages reduced *E. coli* in both the rumen and rectum contents.

4.5.1 Rumen *E. coli*

There are a number of mechanisms that could have been acting to reduce *E. coli* in the rumen. These include: differing levels of VFA production from feeds after fermentation in the rumen; content of anti-microbial coumarins or other compounds in the feeds; the ingestion of different communities of microbes present in the feeds, and; competition between *E. coli* and other rumen microflora.

4.5.2 Volatile Fatty Acid Production

The effect of fasting on *E. coli* growth in the ruminant digestive tract is most probably mediated by a reduction of volatile fatty acid concentration (VFA) and a subsequent increase in the pH of the digestive tract contents (Wolin, 1969; Rasmussen *et al.*, 1993; Duncan *et al.*, 2000). *E. coli* are very sensitive to pH changes (Rasmussen *et al.*, 1993; Duncan *et al.*, 2000). Increased intake of simple and digestible complex carbohydrates generally decreases rumen pH, almost certainly through increased rumen VFA

Table 4.9 Mean (±SED) concentration of E. faecalis (log_{10}counts g^{-1} wet weight) in the rumen and rectum contents of cattle slaughtered in the March and May trial replicates.

	Rumen	Rectum
March	0.46 (±0.17)	0.52 (±0.16)
May	1.36 (±0.17)	2.54 (±0.16)

4.4.3.4 Aerobes and Total Anaerobes

Rumen aerobes and total anaerobe counts (Table 4.7) were strongly affected by dietary treatments ($P<0.001$). The trends for both anaerobes and aerobes appeared very similar, in that meadow hay-fed animals had the highest counts of both aerobes and anaerobes, and maize silage-supplemented haylage and haylage alone had the lowest, in comparison with other treatments (Table 4.7).

Dietary treatments also significantly affected the counts of aerobes and total anaerobes in the rectal contents collected after slaughter (Table 4.7). Fasted, ryegrass hay-fed and pasture fed animals generally had higher counts of anaerobes and aerobes in their faeces, while lucerne hay, haylage and maize silage-supplemented haylage had lower counts (Table 4.7).

4.4.4 Dirtiness scores

There were no significant differences between treatments in the numbers of animals with wet faeces present on the bung (73% of total clean), brisket (67% of total clean), butt (68% of total clean), under the neck (93% of total clean), or overall (63% of total clean). The feet of cattle in the maize silage-supplemented haylage (36% of treatment clean), haylage (43% of treatment clean) and red clover hay (43% of treatment clean) treatments were significantly ($P<0.05$) dirtier than those in the ryegrass hay treatment (86% of treatment clean). However, these treatments did not differ significantly from the other treatments (lucerne hay = 76% clean; meadow hay = 50% clean; fasted = 64% clean; pasture = 71% clean).

concentration, thereby producing conditions unfavourable to the survival of *E. coli* in the rumen (Brownlie and Grau, 1967; Grau *et al.*, 1969; Wolin, 1969; Duncan *et al.*, 2000). With *in vitro* studies, *E. coli* grew best in rumen liquor when the concentration of VFA's was less than 25mM and the pH was 7.2. The growth of the *E. coli* was completely halted when VFA concentration were greater than 75 mM, and growth decreased linearly as pH declined until zero growth was achieved at a pH of 6.0 (Rasmussen *et al.*, 1993). In comparison, VFA concentration in the rumen of healthy, forage-fed animals can reach as high as 150mM (Duncan *et al.*, 2000).

In the present study, rumen pH was relatively high in all treatments, and the range of pH was narrow (about 0.5 pH units). Intuitively, these features do not seem to support a VFA-dependent mechanism. However, mean rumen *E. coli* counts were greatest in fasted animals, which correspondingly had the highest rumen pH at slaughter of all the treatments - pH 7.4, and the lowest intake of VFA precursors in the 48 hours preceding transport. In addition, there was a significant positive correlation between rumen pH and *E. coli*; and significant negative correlations between total intake of either neutral-detergent fibre (NDF) or acid-detergent fibre (ADF), and rumen pH, and; significant negative correlations between total intake of either NDF, or ADF, and rumen *E. coli* counts. This suggests a pH/VFA dependent increase in *E. coli* was in effect in the rumen.

NDF and ADF are the major determinants of the dry matter content in the rumen (Holmes *et al.*, 1984). This may explain why in the present study, a significant negative correlation was determined between increasing dry matter % of rumen contents and rumen *E. coli* counts.

Haylage and red clover hay treatments had the lowest mean rumen counts of *E. coli*. Haylage and red clover hay treatments also had the highest and third highest mean intakes of NDF, were the most digestible of the conserved forages and had the lowest mean rumen pH, suggesting a greater VFA production than other feeds. Haylage and silage contain lactate (White *et al.*, 1999). Lactate is more strongly acidic than the rumen VFAs acetate, propionate and butyrate, and is a more potent reducer of rumen pH (Goff and Horst, 1997).

This suggests that lactate consumed in haylage in the present trial may also have contributed directly to VFAs and the acidity of the rumen. It seems highly likely that in this study, despite the post-transport fasting period that may have increased rumen pH at slaughter, that the carry-over ruminal VFAs and lactate from the pre-transport diet of animals in these treatments were probably sufficiently elevated, and pH sufficiently lowered, to cause a suppression of rumen *E. coli* counts at slaughter.

Ryegrass hay-fed animals in the May replicate also had a high mean dietary fibre intake, although the mean rumen pH was not especially low, and rumen *E. coli* counts were greater than those determined for other conserved feed treatments. This may be due to the poor quality of that feed. Lignin is an indigestible form of dietary fibre that increases in forage plants as they mature (Holmes *et al.*, 1984). The ryegrass hay used in the trial was grown as a near-pure sward, and the hay cut after harvesting for ryegrass seed, and so the sward consisted of very mature plants. Lignin may have made up a significant proportion of dietary fibre in this feed, which could reduce the proportion of digestible fibre substrates from which VFAs could be produced. This is supported by the low organic matter digestibility of this feed, and may also explain the palatability problem encountered in the March replicate of this treatment.

Lucerne hay and meadow hay treatments both had fairly similar NDF intakes to pasture-fed animals and comparable organic matter digestibilities to March pasture (between 55-60% organic matter digestibility). Mean rumen pH of meadow hay-fed cattle was also very similar to pasture and ryegrass hay-fed animals, while the rumen pH of lucerne hay-fed animals (pH 7.2) was more comparable to that of fasted animals (pH 7.4). Consequently, based on the above arguments, it could be expected that the mean rumen *E. coli* counts of these four treatments would be similar. However, the mean rumen *E. coli* counts of lucerne hay and meadow hay animals appeared to be somewhat lower than that of either pasture or ryegrass fed animals. It seems likely that for both these feeds factors other than VFA production may have been involved in suppressing rumen *E. coli*. Lucerne has a high buffering capacity and maybe an outlier in terms of pH.

4.4.3 Coumarins

It may be that coumarins, a diverse group of benzopyrone compounds found in some plants, could have contributed to anti-microbial activities of the lucerne and meadow hays fed to cattle in this study. Lucerne (*Medicago sativa* L.) contains at least nine different types of coumarins in the aerial parts of the plant (Murray *et al.*, 1982). One of these, esculetin, has been shown *in vitro* to have a growth-suppressing effect on commensal strains of *E. coli* and on *E. coli* O157:H7 incubated in rumen fluid, and this effect was greatly enhanced by the addition of 100mM of VFAs (Duncan *et al.*, 1998). Other coumarins have also been reported as having anti-microbial properties (Murray *et al.*, 1982; Duncan *et al.*, 1998).

The meadow hay fed in the present study contained many plant species that are known to contain coumarins; they were *Trifolium repens, Trifolium pratensis, Polygonium aviculare* and *Taraxacum officinale* – the latter containing the aforementioned, "anti-*E. coli*" coumarin, esculetin. Red clover (*Trifolium pratensis*) contains the coumarin "coumestrol", although this coumarin is more renowned for its oestrogenic than its anti-microbial activity. In contrast, perennial ryegrass (*Lolium perenne*) currently has not been recognised as containing any of the, approximately 1000, known coumarin compounds.

When feed treatments in the present study were ranked by expected coumarin intakes (based on the expected coumarin concentration of plant species represented from Murray *et al.*, 1982, and the quantity of these plants eaten in the 48 hours before transport), there was a significant negative relationship between feed coumarin rank and *E. coli* counts in the rumen. This effect was still present to a lesser degree when red clover hay was excluded from the analysis (because its coumarins are known to be oestrogenic rather than anti-microbial). The coumarins rank *E. coli* negative correlation was also present in the faeces of the cattle. These findings support the possibility that coumarins, in combination with VFA's, may have contributed to the lower ruminal and faecal *E. coli* counts of lucerne- and meadow hay-fed animals, particularly when compared to those animals fed ryegrass hay or ryegrass-dominant autumn pasture. Further studies that include the objective measurement of coumarin intake are required to validate this theory, and could ultimately lead to the

targeted feeding of specific types of herbage species before slaughter for a dual action against potential food pathogens in the digestive systems of cattle.

4.5.4 Competition from other microbes

E. coli are generally not considered a predominant species of microbe in the rumen (Stewart *et al.*, 1997). It is possible that more prevalent species of microbes in the rumen could suppress the growth of rumen *E. coli* through mechanisms such as competition for nutrients (Duncan *et al.*, 2000). Enterococci have been reported as having a suppressive effect on the growth of *E. coli* (Franz *et al.*, 1999). *E. faecalis* and *E. faecium* tend to be the predominant enterococci species in the digestive tract of humans and livestock, with *E. faecalis* being the enterococcus species more commonly associated with cattle. Both of these species of enterococci have been used in probiotic preparations for humans and farm animals (Franz *et al.*, 1999). Consequently, in the present study *E. faecalis* were isolated and enumerated from enterococci plates. However, *E. faecalis* were not well represented in the digestive systems of the trial cattle - being undetected in 59 of the 112 rumen contents samples, and 34 of the 112 faecal samples. Overall, *E. faecalis* accounted for less than 20% of the total ruminal enterococci, and about 37% of the faecal enterococci. It is possible that much of the putative generic enterococci population may have been *Streptococcus bovis*. This species is prevalent in the rumen of cattle over a relatively wide physiological pH range (Stewart *et al.*, 1997). It also has similar colony morphology to enterococci when cultured on the KF media used for enumeration of enterococci in the present study (Kenner *et al.*, 1961; Franz *et al.*, 1999).

If competition by rumen microbes was responsible for suppression of *E. coli* numbers in the rumen, a significant negative correlation between the numbers of those microbes and *E. coli* would be expected. However, the counts of generic rumen enterococci and *E. faecalis* (when present) were not significantly related in the rumen. There were also no significant negative relationships between rumen aerobes, or anaerobes and rumen *E. coli* numbers. This suggests that, in this study, competition from other microbes in the rumen such as

enterococci was not one of the major mechanisms responsible for the suppression of rumen *E. coli* seen with some of the feed treatments.

It also indicates that although the feeds contained large counts of *E. faecalis*, aerobes and anaerobes, the ingestion of the relatively large quantities of these bacteria did not promote competitive suppression of *E. coli* in the rumen. Likewise, the ingestion of *E. coli* in the trial feeds was negligible, suggesting *E. coli* was already present in the digestive tracts of trial cattle before the start of the feeding treatments.

4.6.5 Faecal *E. coli*

The relationship between pH and *E. coli* in the faeces of fasted animals was not clear in the present study. However, it is probable that the high counts of faecal *E. coli* in this study were related to a greater number of *E. coli,* on a concentration basis, exiting from the rumen into the lower reaches of the digestive tract (as seen in the previous chapter).

Significant positive correlations were detected between faecal *E. coli* and aerobes and anaerobes, respectively. As the relationships were positive rather than negative, they cannot be considered as indicative of competitive suppression. It is more likely that these positive relationships occurred as the increases in *E. coli* numbers down the digestive tract began to comprise a relatively greater proportion of the counts of both aerobes and anaerobes.

Overall, faecal *E. coli* counts were less strongly influenced by dietary treatments than the rumen *E. coli* counts. Although fasted animals' faecal *E. coli* were still significantly greater than fed-treatments, statistical differences between the feed treatments were not detected. It is possible that the digestive characteristics of the different feeds, and the interactions of these characteristics with the 16 hours post-transport fasting during overnight lairage at the abattoir, confounded feed treatment differences in *E. coli* counts in the rectum. Jordan and McEwan (1998) found that changing feedlot cattle diets to high roughage feed for four days reduced faecal *E. coli*. However, when subsequently fasted for 48 hours, faecal *E. coli* counts were higher in the roughage-fed cattle than those of similarly fasted normal

concentrate-fed cattle. Although in the present trial, the 16-hour post transport period of fasting was not as long as that in Jordan and McEwan's (1998) study, it may have been long enough to promote increased faecal *E. coli* through a reduction of the flow of VFA substrates into the colon. This would vary for each feed type, being dependent on characteristics such as digestibility, fibre length and mean rate of passage.

4.5.6 Welfare and Production

The manipulation of pre-slaughter cattle management can affect factors other than gut microbiology that are also considered important in a commercial operation. Pre-slaughter management can affect animal stress and dehydration, and thus welfare. Stress and dehydration can also detrimentally affect the production parameters carcass weight and meat quality (meat stickiness, pH and associated quality defects) (Gregory, 1998). Dietary treatments investigated in our trial did not detrimentally affect stress at slaughter (as suggested by a lack of treatment effects on plasma cortisol, a commonly used indicator of stress (Gregory 1998)), meat pH, stickiness or the incidence of blood splash. Plasma protein values suggested that haylage-feeding might have improved cattle hydration at slaughter in comparison to fasting, or feeding meadow hay or pasture, before transport.

Another benefit of feeding cattle before transport to slaughter as opposed to fasting may be the protection of carcass weight – the mean carcass weight of fed heifers in the present study was 5kg heavier than that of the fasted heifers. The maintenance of carcass weight when cattle are fed hay or pasture silage, in comparison to fasting, before transport to slaughter has been reported in previous studies conducted at MIRINZ. Carcasses of Friesian bulls fed meadow hay or haylage for up to 20 h before transport for slaughter were slightly, but significantly, heavier than those fasted for the same length of time (Jacobson and Cook, 1997; Jacobson *et al.*, 1998). A simple cost-benefit assessment undertaken in one of these studies, in the economic climate of the time, showed a positive margin increase of about $NZ 15-20 per bull after the cost of the feed was taken into account (Jacobson *et al.*, 1996). Further cost-benefit analyses of pre-transport feeding, taking into account

carcass weight gains and reduced carcass contamination risks, versus possible increased truck cleaning costs, may be indicated.

4.6 CONCLUSION

Fasting for 24 hours before transport and overnight lairage significantly increased rumen and faecal *E. coli* at slaughter. This suggests that extended periods of fasting in commercial cattle slaughter operations should be avoided where possible. Red clover hay and en-silaged pasture (haylage), were the most effective pre-transport diets for reducing *E. coli* counts in the rumen. This was most probably a consequence of increased production of *E. coli*-toxic volatile fatty acids, and additionally for haylage, the direct ingestion of lactic acid produced during fermentation in the haylage bale. The reduction of ruminal and faecal *E. coli* in lucerne hay-fed, and to a lesser degree, in meadow hay-fed heifers, may also be coumarin associated.

The results of the present study lead to the following two recommendations for the management of finished beef cattle raised on pasture: 1) Feed red clover hay for 48 hours prior to transport to slaughter, to reduce *E. coli* counts in the rumen to less than 1 log count g^{-1}. 2) Reduce preslaughter fasting to 18 hours or less, including transport, to minimize gastrointestinal *E. coli* counts at slaughter and to reduce losses in carcass weight.

Chapter 5

Effects of rearing chicks in the presence of adults on their responses to a fearful situation and their ability to withstand *Eimeria tenella* infection

5.1 ABSTRACT

The effectiveness of a natural probiotic system provided by the presence of adult birds was compared with a commercially available prebiotic and synbiotic on the resistance to infection with *Eimeria tenella* in chicks. The prebiotic used in this study was Bio-Mos® (Alltech, USA), and the synbiotic, Acid Pak 4-Way® (Alltech, USA). The combination of commercial prebiotic (Bio-Mos®) and synbiotic (Acid-Pak-4-Way®) caused increased *Eimeria tenella* infection with the presence of hens imparting partial resistance in chicks, but these effects were not reflected in differences in chick growth rates or weights, which were generally higher for the group receiving the commercial additive and for the group without hens.

The effect of rearing chicks with and without adult birds on their ability to adapt to a fearful situation, and on growth rate of chicks were also investigated. In modern farming systems, chicks are not raised with their parents. This is an unnatural situation. Chicks reared with hens appeared to be less stressed, as exhibited by a shorter freezing response to the open field test and a shorter delay to vocalisation in the first and third week of testing, compared to chicks reared without hens. The trend was reversed during the second week with the chicks reared with hens being more stressed. This time of greater apparent stress was within a period when they grew significantly more slowly than the no-hen group. This led to the chicks reared without hens being heavier at 25 days. There were no significant effects of hens on spontaneous behaviour and startle responses of the chicks.

5.2 INTRODUCTION

5.2.1 Coccidiosis

Coccidiosis is a protozoan disease of fowl and is a problem in all poultry-producing areas. It is characterised by diarrhoea and variable mortality. The most commonly recognised coccidia infecting chickens and other poultry are of the genus *Eimeria* (McDougald, 1998). *Eimeria tenella* is one of the most common and pathogenic coccidia of domestic poultry with a worldwide distribution (Soulsby, 1969). The *Eimeria* infecting chickens and turkeys do not infect other animals, nor do those of other animals infect poultry (McDougald, 1998). Despite recent advances in control and treatment, the disease remains one of the principal causes of economic loss to the poultry industry.

Coccidiosis usually occurs in growing birds and young adults. It is seldom seen in birds under 3 weeks of age unless they are brooded on contaminated litter. It is possible to infect day-old chicks (Soulsby, 1969). Old birds are usually immune because exposure during early life is difficult to avoid.

Eimeria tenella inhabit the caeca and adjacent intestinal tissues causing a severe disease characterised by bleeding, high morbidity and mortality, lost weight gain and emaciation (McDougald and Reid, 1991). Clinical caecal coccidiosis is produced only when heavy infections are acquired over a relatively short period of time not exceeding 72 hours (Soulsby, 1969).

Signs of coccidiosis outbreak are usually general. Seventy-two hours after infection it is first noticed that chickens droop, cease feeding and huddle to keep warm (Soulsby, 1969). After 96 hours blood appears in the droppings and on the fifth and sixth day the greatest haemorrhage occurs (Soulsby, 1969). Affected birds become pale and droopy, tend to huddle, consume less feed and water, have diarrhoea and may become emaciated and

dehydrated. Chickens in the acute phase of caecal coccidiosis have ballooned caecal pouches full of free blood.

By the $8^{th} - 9^{th}$ day the bird is either dead or on the way to recovery. Mortality is the highest between the 4^{th} and 6^{th} day with death sometimes occurring unexpectedly due to excessive loss of blood (Soulsby, 1969). In the recovery stage, cheesy cores tinged with variable amounts of blood are present in the caecal pouches. In birds that have recovered from the acute disease, a chronic illness may develop as a result of a persistent caecal core which is usually expelled about 14 days after infection (Soulsby, 1969).

The number of infective coccidia that a bird eats determines whether an infection will be mild enough to go unnoticed or severe enough to cause visible illness. Under ordinary farm or poultry rearing conditions, it is likely that all birds are exposed to infection, but the severity of the disease is dependent, very largely, on the number of oocysts ingested (Soulsby, 1969; McDougald, 1998).

5.2.2 Prevention of coccidiosis

Since the 1950's, anticoccidial drugs have been used to control coccidiosis in broilers (McDougald, 1998). The use of these drugs however has not eliminated outbreaks of coccidiosis in poultry. Outbreaks of coccidiosis are caused by various conditions, including failure of the anticoccidial, either through drug resistance of the coccidia or inadequate spectrum of activity of the drug (McDougald, 1998). Other possible problems include concurrent disease that might destroy the birds' immune system or interfere with coccidiostat intake by reduction in feed or water consumption, and the mistaken diagnosis of coccidiosis when other diseases cause the same type of lesions (McDougald, 1998).

It is difficult, if not impossible to prevent coccidiosis by sanitation practices alone. In natural conditions, the newly hatched chick is quickly inoculated with intestinal microorganisms from such sources as parental contact and adult faeces on litter and food. An important consideration in poultry farming is, the present practice of rearing chickens

away from adult birds, as this reduces pathogen loading. Hygienic rearing conditions reduce the opportunity for the chicks to acquire an indigenous intestinal flora that might influence pathogen survival and proliferation in the gut (Lloyd *et al.*, 1977).

The incubator hatched chicken is an example of an animal which has been deprived of its characteristic protective gut microflora by the artificial environment imposed upon it (Fuller, 1999). It emerges from the egg into a clean hatchery totally removed from any contact with its mother or any adult chickens. It therefore acquires its gut microflora from its immediate environment which does not contain all the microorganisms required to confer protection on the newly hatched chick (Fuller, 1999). Modern hygienic practices in poultry sheds, while admirable for control of many poultry pathogens, may result in a delay in the development of a stable indigenous flora of the intestine (Nurmi and Rantala, 1973; Lloyd *et al.*, 1977). This lack of indigenous flora could deny the host part of its natural defence mechanism against opportunistic enteric pathogens.

5.2.3 Prebiotics, probiotics and synbiotics

There is increasing interest in the possibilities of manipulating the composition of the gut microflora by foods or food ingredients. Attempts have been made to improve health status by modulating the indigenous intestinal flora by live microbial adjuncts, called probiotics (Holzapfel, 1998). There are two approaches to increasing the number of health-promoting organisms in the gastrointestinal tract.

The first approach is the oral administration of live beneficial microbes, termed probiotics. Probiotics are defined as mono- or mixed cultures of live microorganisms which, when applied to animal or humans, beneficially affect the host by improving the properties of the indigenous microflora (Fuller, 1989; Holzapfel, 1998; Walker and Duffy, 1998, Crittenden, 1999; Short and Merryfield, 1999). Survival of probiotic organisms in the gut depends on their possessing colonisation factors which enable them to resist the antibacterial mechanisms (chemical and physical) which operate in the gut (Fuller, 1989).

The second approach is to selectively modify the composition of the microflora using dietary supplements called prebiotics. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health (Walker and Duffy, 1998; Short and Merryfield, 1999; Crittenden, 1999). A common aim with prebiotics is to promote the proliferation of bifidobacteria and endogenous lactic acid bacteria in the colon (Crittenden, 1999; Walker and Duffy, 1998). They can have other effects, such as the modulation of lipid metabolism (Walker and Duffy, 1998).

The concept of 'synbiotic' has been proposed to characterise food additives with prebiotic and probiotic properties as health enhancing functional foods (Walker and Duffy, 1998).

This study compared the effectiveness of a natural probiotic system that is provided by the presence of adult birds with a commercially available prebiotic and synbiotic on the resistance to infection with *Eimeria tenella*. The prebiotic used in this study was Bio-Mos® (Alltech, USA), and the synbiotic, Acid Pak 4-Way® (Alltech, USA). Bio-Mos® is a mannanoligosaccharide derived from the cell wall of the yeast, *Saccharomyces cereviciae*. Mannanoligosaccharides are not degraded by the intestinal microflora, however, they provide receptor sites to which pathogenic bacteria bind. In this manner they help to prevent colonisation of the gastrointestinal tract by pathogenic bacteria. Mannanoligosaccharides are thought to inhibit binding of certain pathogenic bacteria to the lining of the digestive tract, while acting as a nutrient to other beneficial bacteria. Researchers have discovered that bacterial pathogens, such as *Escherichia coli*, salmonella and clostridia will also attach to mannanoligosaccharides (MOS) (Cole and Close, 1999). MOS are complex, non-digestible carbohydrates contained in yeast cell walls (Cole and Close, 1999). If the pathogen attaches to the MOS, it looses the ability to infect the intestinal cell and is excreted from the body in the faeces (Cole and Close, 1999). The animal does not suffer any digestive disturbance or infection.

Acid-Pak-4-Way® contains an organic acid blend, electrolytes, digestive enzymes and lactic acid producing bacteria. Organic acids lower the pH of the intestinal material

inhibiting the growth of pathogenic bacteria and as a consequence there is an improvement in gastrointestinal health which results in enhanced growth performance and improved feed utilisation (Cole and Close, 1999). Even at low concentrations, acids reduce the pH of the feed and inhibit the growth of certain pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Cole and Close, 1999). Organic acids also inhibit growth of moulds, which can produce mycotoxins that depress the pig's immune system (Cole and Close, 1999).

The lactic acid bacteria are still the most commonly used probiotic microorganisms. They are attractive because they are known to be involved in control of the gut microflora and because they are non-pathogenic (Fuller, 1993).

5.2.4 Behaviour of chicks

This study also examined the effect of rearing chicks with and without adult birds on their ability to adapt to a fearful situation, and on growth rate of chicks. In modern farming systems, chicks are not raised with their parents. This is an unnatural situation. In the wild, survival depends greatly on rapid bonding between the hen and her chicks but an important question not fully answered is the effect which artificial hatching and rearing has on subsequent hen behaviour in such traits as feeding and nesting (Kilgour and Dalton, 1984). On the other hand, the chick's precocity allows it to adapt to rearing without a broody hen in intensive systems (Kilgour and Dalton, 1984).

The intensity of fear responses in chicks increases from very low levels on day 1 to an early peak on day 2 or 3 (Andrew and Brennan, 1983). This is followed by a trough, a larger peak on days 5 and 7, a trough on day 8, and a final even larger rise to sustained high values from day 10 (Andrew and Brennan, 1983). Roden and Wechsler (1998) found several results indicating that chicks housed without a hen may be more fearful than chicks raised by a hen. Separation from the social companions becomes an important social factor in eliciting calling in chicks with social experience (Kaufman and Hinde, 1961).

The open field test, in which animals are exposed individually to a novel arena, was employed in the current study to estimate levels of fear in the chicks. Results gained from the open field test are open to a variety of interpretations, since fear related behaviour in the open field may involve arousal, predator defence, social reinstatement, exploration and territorial marking (Lowndes and Davies, 1996). For behaviour to be examined at fear levels intuitively regarded as higher than those induced by the open field alone, chicks have to be exposed to additional startling stimuli (Jones, 1987). After a brief, though variable period of acclimatisation to an open field or novel environment the bird may be presented with a sudden auditory or visual stimulus and observed over a further period (Jones, 1987). Exposure to such a startling stimulus generally elicits strong fear reactions thus enhancing passive behaviour and decreasing activity and vocalisation (Jones, 1987). In the present study a startle reponse test was performed immediately after the open field test as a complement. The advantages and disadvantages of parental presence when rearing chicks concerning disease resistance, fearfulness and growth rate will be investigated.

5.3 MATERIALS AND METHODS

5.3.1 Animals and Treatments

Three weeks before the arrival of the day-old male layer chicks (-21 days), 15 end-of-lay ex-battery hens, approximately 70 weeks old, were transported from a layer facility to the experimental facility. The hens were kept on untreated wood shavings in a pen with feed and water provided *ad libitum*, and allowed free access to outside pasture. On -20 days they were treated with a coccidiostat, Baycox®, administered through drinking water for 48-60 hours, and then dosed with 1ml directly into the crop on –10 days. This was to ensure, as much as was possible, that the hens were free of coccidia before the introduction of the chicks.

The day before the chicks arrived the hens were randomly allocated to three separate pens out of a total of six pens (three hens per pen). Each pen was 3 x 4 m, fully lined with plastic to stop cross contamination and the floor was covered with untreated wood

shavings. Nesting boxes were available in each pen. The three remaining pens were identical apart from the nesting boxes. The light cycle was 12h light:dark ratio, with the lights on at 8 a.m. and off at 8 p.m. Brooder lamps (250W) were set up in each pen to ensure that the chicks were kept at the optimum temperature of 30° C (Kilgour and Dalton, 1984). As the hens were unused to chicks and therefore not overly maternal it was felt that for the survival and comfort of the chicks, brooders should be available in these pens.

On day 1, 312 day-old male layer chicks were transported from the hatchery to the experimental facility. On arrival they were weighed on a set of electronic scales (Texas Instruments XL-6100, e=0.1g) and randomly separated into six treatment groups. The six groups were:

Chick control: chicks only and no coccidia challenge

Hen control: chicks and hens and no coccidia challenge

Eimeria control: chicks only and coccidia challenge

Natural probiotic: chicks and hens and coccidia challenge

Commercial: chicks, commercial prebiotic and synbiotic and coccidia challenge

Nat/Comm: chicks and hens, commercial prebiotic and synbiotic and coccidia challenge.

Each treatment group contained at least 50 chicks (Hen control 52, Chick control 53, Natural probiotic 51, Eimeria control 51, Nat/Comm 50, and Commercial 51). Fifteen chicks in each treatment were randomly allocated numbers (1-15) and were numbered with a marker pen on their back and head for individual identification. An additional 5 chicks from each treatment were sprayed with purple stock marker (Donaghys Super Sprayline Marker). The Hen control, Natural probiotic and Nat/Comm treatment groups each had two hens present (Figure 5.1). This was reduced to one hen in the Natural probiotic treatment group on day 7 (after introduction of chicks).

Figure 5.1 Hens and chicks from the Hen control treatment group.

Poultry mash containing no coccidiostat (Seales Ltd, Morrinsville, New Zealand) was fed *ad libitum* to each treatment group with the Commercial and Nat/Comm groups additionally receiving Bio-Moss® (a mannanoligosaccharide derived from the cell wall of the yeast, *Saccharomyces cereviciae*, Alltech, U.S.A.) with their mash (2g/kg of mash). Water was available *ad libitum* with the Commercial and Nat/Comm treatment groups additionally receiving Acid-Pak-4-Way® (Acid-Pak-4-Way® contains an organic acid blend, electrolytes, digestive enzymes and lactic acid producing bacteria, Alltech, U.S.A.) in their drinking water (0.5g/litre of water).

5.3.2 Weight of chicks

The chicks were weighed on days 1, 3, 5, 7, 11, 15, 18 and at slaughter on either day 24 or 25. Each chick was individually weighed using a set of electronic scales (Texas Instruments XL-6100, e=0.1g) (Figure 5.2). All chicks from a treatment group were caught and placed in a plastic storage bin and kept under a heat lamp while awaiting weighing. Weighing of each treatment was fully completed before the next was started. A different bin was used for each treatment.

Figure 5.2 Individual weighing of chicks.

5.3.3 Behaviour

5.3.3.1 Open Field Test

On days 2, 9 and 16, twenty chicks from each treatment group (number 1-15 and 5 purple) were tested for latency to movement and vocalisation in an open-field test followed immediately by a startle response test. The open-field was a circular pen, one metre in diameter with a height of 0.6m (Figure 5.3).

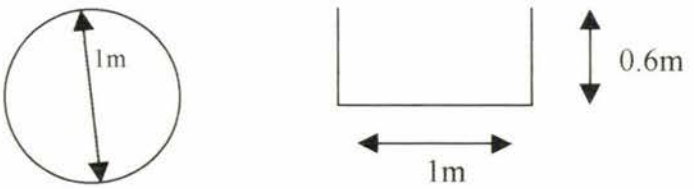

Figure 5.3 Dimension of open field.

The base of the pen was not attached to the walls to allow for ease of cleaning after each treatment group. The interior was white with the floor covered in white paper that was changed after each treatment group. One treatment group was tested at a time. Each chick was placed individually into the centre of the pen and time to first movement and first vocalisation was recorded.

5.3.3.2 Startle Response

After 2 minutes in the open field pen the chick was then startled and the first reaction was noted. On days 2, 9 and 16, chicks were startled by dropping a novel object into their field of vision (blue glove, suction dart gun and koosh® ball respectively). Each chicks' first reaction was noted.

5.3.3.3 Scan

Scan behaviour of the 15 marked chicks in each treatment group was recorded on days 4, 6, 8, 10, 14, 22 and 23. On every recording day the chicks were watched from 9am –11am and 2pm – 4pm, with a scan of each group every five minutes. This allowed 8 recordings for each treatment group each day. The number of chicks performing each behaviour at a given time was recorded. The behaviours recorded were eat, drink, sleep, walk, stand, sit and other (scratch, dust bathing, preen, run, run/flap, flap and interactions).

5.3.4 *Eimeria tenella* Challenge

On day 19, the chicks from the Eimeria control, Natural probiotic, Commercial and Nat/Comm treatment groups were direct dosed into the crop with 10 000 *Eimeria tenella* oocysts. The oocysts were diluted from the original 135 000 oocysts per ml with distilled water to give a dose of 10 000 oocysts per ½ ml.

On day 24 (5 days post coccidia dosing) chicks numbered 11-15, the purple marked and 15 others from each treatment group (randomly chosen) were slaughtered by cervical dislocation and lesion score was obtained. The remaining chicks (numbered 1-10 and others) were slaughtered the following day (day 25 or 6 days post coccidia dose). Immediately before slaughter each chick was weighed. Dissection was completed immediately following death and the caeca of each bird scored for lesions. Lesions were scored on a 5-point scale (0 = no lesions to 4 = maximum lesions) using the method as

described by Johnson and Reid (1970) in Conway (1979). Blood was collected from the heart of the numbered birds from each treatment following cervical dislocation. The brains of the numbered birds were also collected following lesion scoring.

5.3.5 Statistical Analysis

Statistical analyses were performed using analysis of variance (ANOVA) and Chi-square using SigmaStat version 2 (Jandel Scientific Software, U.S.A). The general least squares procedure from SAS (S.A.S. Institute Inc., U.S.A.) was used for analysing scan behaviour.

Weight data and growth rate were analysed for each day by a one-way ANOVA (model: $y_{ij} = \mu + t_i + e_{ij}$; where t_i is the effect of the i^{th} treatment), with the six individual treatment groups. Weight data, up to *Eimeria tenella* challenge, was also analysed by two-way ANOVA (model: $y_{ij} = \mu + t_i + u_j + tu_{ij} + e_{ij}$; where t_i is the effect of presence or absence of hens and u_j the effect of commercial additive). After the challenge with *Eimeria tenella*, the weight data and lesion scores were analysed using a two-way ANOVA with hen treatment (presence or absence of hens) and eimera challenge with or without commercial additive as the cross-classification variables.

Open field behaviour data (time to movement and time to vocalisation) was analysed within each test day by a one-way ANOVA (model: $y_{ij} = \mu + t_i + e_{ij}$; where t_i is the effect of the i^{th} treatment), with 2 treatments (presence/absence of hen). The presence/absence of a commercial additive was originally included in the statistical model, but was not retained because it did have significant effects on any of the characteristics.

Scan behaviour was analysed as percentages of chicks performing a certain behaviour with two-way repeat measures ANOVAs (model: $y_{ijk} = \mu + t_i + u_j(t_i) + v_k + tv_{ik} + e_{ijk}$ where t_i is treatment (with-hen vs no-hen); u_j pens (3 per treatment) and v_k days (5 days of measurement) for days 4-14. Within the analysis of variance, treatment effects were tested against pen within treatment, and day and day-by-treatment interaction effects were tested

against the overall error. Data for days 22 and 23 (after *Eimeria tenella* challenge) were not analysed because of the low number of samples per treatment group.

The Tukey Test was used for all pairwise comparisons of the mean responses to the different treatment groups. The Tukey Test controls the errors of all comparisons simultaneously.

Chi-square analysis was used to analyse the startle response data to determine the effect of presence or absence of hens on a behavioural response. The model used was $\chi^2 = (n-1)s^2 / \chi^2$; where s^2 is the sample variance which is an unbiased estimate of the population variance, χ^2. The smaller the resulting P value, the greater the probability that the samples are drawn from populations with different distributions among the categories. The chi-sq significance analysis tests whether the pattern of numbers in the different movement categories differs between the 2 treatments (presence or absence of hens) on the 3 days of measurement.

5.4 RESULTS

5.4.1 Weight of chicks

Significant differences between hen and no-hen groups in live weight were shown from day 3 ($P<0.05$, Tables 5.1 and 5.2). The treatment groups receiving the commercial additives (Commercial and Nat/Comm) were heavier than corresponding treatment groups without the additive (Figure 5.4). These differences were significant on days 11, 15 and 18 ($P = 0.006$, <0.001 and <0.001 respectively). The interaction between presence of hens and commercial additives was not significant. The results were similar for the fifteen numbered chicks per treatment (Tables 5.3 and 5.4).

The overall average daily gain for each treatment group followed the same trend, with the no-hen groups having the highest average daily gain (Table 5.5). This result was consistent

with results from the subset of subjects that were individually numbered in each treatment group.

The average daily gains showed significant differences between the no-hen and hen groups from days 7 to 15 ($P<0.05$). During this period of weight gain, chicks reared with hens lagged behind the chicks reared by themselves. The Hen control, Natural probiotic and Nat/Comm treatment groups average daily gains' all decreased over this time period whereas the Chick control, Eimeria control and Commercial groups increased (Table 5.6, Figure 5.5). The addition of the commercial additives also significantly affected the average daily gain of chicks from days 7 to 15 ($P<0.05$). The interaction between presence of hen and additive was not significant.

Table 5.1 Effect of presence or absence of hen and presence or absence of commercial additive on average weight of all chicks (grams ± sem) on the 7 days when they were weighed prior to the *Eimeria tenella* challenge.

	Hen treatment			Additive treatment		
Day	Hen	No hen	P	Additive	No additive	P
1	38.66 ± 0.28	38.60 ± 0.28	0.88	38.71 ± 0.33	38.54 ± 0.23	0.67
3	43.68 ± 0.28	44.44 ± 0.28	0.05	44.40 ± 0.32	43.72 ± 0.22	0.08
5	51.12 ± 0.39	54.59 ± 0.39	<0.001	53.25 ± 0.45	52.47 ± 0.31	0.15
7	60.86 ± 0.48	66.15 ± 0.48	<0.001	63.61 ± 0.56	63.34 ± 0.39	0.75
11	77.52 ± 0.67	92.13 ± 0.67	<0.001	86.13 ± 0.78	83.52 ± 0.54	0.006
15	92.11 ± 0.98	120.59 ± 0.98	<0.001	109.28 ± 1.13	103.42 ± 0.79	<0.001
18	110.32 ± 1.27	143.09 ± 1.26	<0.001	130.37 ± 1.47	123.04 ± 1.01	<0.001

Table 5.2 Means (± sem) for the final weight (grams) and the growth rate between days 18 and 24 for chicks with or without hens present (with-hens vs no-hens), and for groups receiving the Eimeria challenge (E) with or without the commercial additive (Add).
Interactions between the hen effect and the E or Add treatments were not significant ($P > 0.05$). Means without a common superscript letter are significantly different ($P < 0.05$).

	Hen treatment			Eimeria/additive treatment			
	With hen	No hen	P	Control	E	E + Add	P
Number of chicks	153	155		105	102	101	
Final weight	156.2 ± 2.0	205.0 ± 2.0	<0.001	180.0 ^{a} ± 2.5	172.5 ^{a} ± 2.5	189.2 ^{b} ± 2.5	<0.001
Growth rate	7.84 ±0.4	10.54 ± 0.4	<0.001	9.70 ^{b} ± 0.4	8.06 ^{a} ± 0.4	9.81 ^{b} ± 0.4	0.007

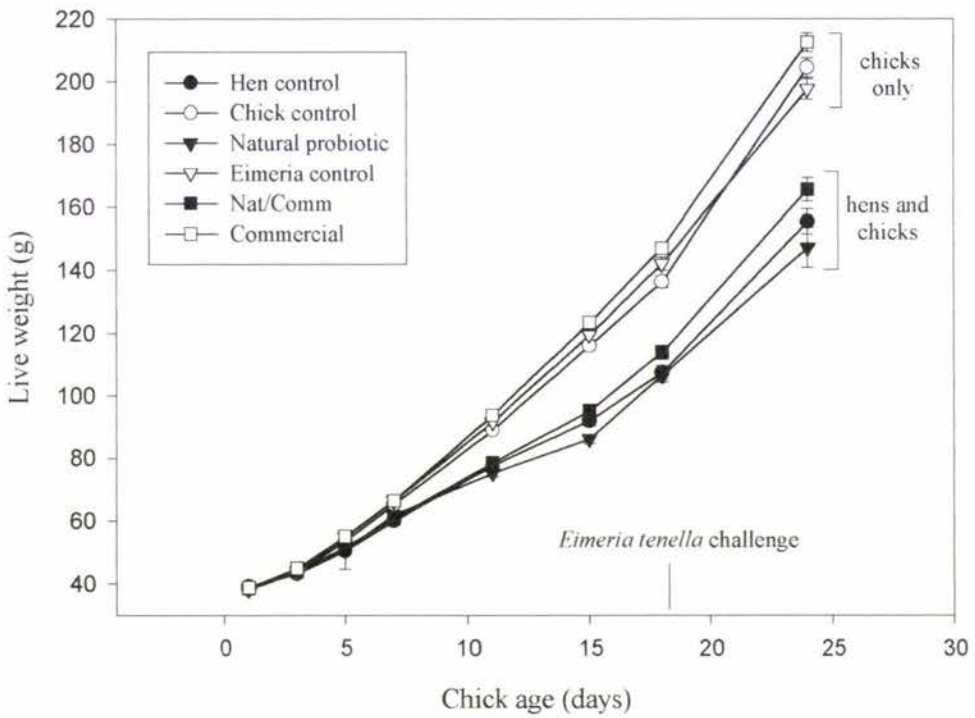

Figure 5.4 Average chick live weight (± sem) per treatment group.

Table 5.3 Effect of presence or absence of hen and presence or absence of commercial additive on average weight of 15 numbered chicks (grams ± sem) on the 7 days prior to the *Eimeria tenella* challenge.

Day	Hen	No hen	P	Additive	No additive	P
1	38.46 ± 0.59	38.92 ± 0.59	0.58	39.40 ± 0.68	37.98 ± 0.48	0.09
3	43.68 ± 0.58	44.68 ± 0.58	0.23	44.83 ± 0.67	43.52 ± 0.47	0.11
5	51.20 ± 0.81	54.90 ± 0.81	0.002	53.97 ± 0.93	52.13 ± 0.66	0.11
7	61.61 ± 0.95	66.64 ± 0.95	<0.001	64.76 ± 1.10	63.49 ± 0.78	0.35
11	78.40 ± 1.34	92.78 ± 1.34	<0.001	87.63 ± 1.54	83.56 ± 1.09	0.03
15	93.18 ± 1.79	121.38 ± 1.79	<0.001	111.20 ± 2.07	103.37 ± 1.46	0.003
18	113.16 ± 2.42	143.80 ± 2.42	<0.001	133.14 ± 2.80	123.82 ± 1.98	0.008

Table 5.4 Means (± sem) for the final weight (grams) for the 15 numbered chicks with or without hens present (with-hens vs no-hens), and for groups receiving the *Eimeria* challenge (E) with or without the commercial additive (Add).
Interactions between the hen effect and the E or Add treatments were not significant ($P > 0.05$). Means without a common superscript letter are significantly different ($P < 0.05$).

	Hen treatment			Eimeria/additive treatment			
	With hen	No hen	P	Control	E	E + Add	P
Number of chicks	45	45		30	30	30	
Final weight	160.3 ± 3.8	202.1 ± 3.8	<0.001	179.0 ^{ab} ± 4.7	173.3 ^{b} ± 4.7	191.3 ^{a} ± 4.7	0.02

Table 5.5 Influence of hens on overall average daily gain of chicks from one to twenty-five days of age.

Means within the same column without a common superscript letter are significantly different ($P<0.05$)

Treatment	Average Daily Gain (g/day) (mean ± sem) all chicks per treatment	Average Daily Gain (g/day) (mean ± sem) 15 numbered chicks per treatment
Hen control	4.9^{bc}±0.2	4.8^{c}±0.3
Chick control	6.9^{a}±0.1	6.9^{a}±0.3
Natural probiotic	4.3^{c}±0.4	4.7^{c}±0.3
Eimeria control	6.6^{a}±0.1	6.5^{ab}±0.3
Nat/Comm	5.3^{b}±0.2	5.7^{bc}±0.2
Commercial	7.2^{a}±0.1	7.0^{a}±0.2

Table 5.6 Effect of presence or absence of hen and presence or absence of commercial additive on average daily gain (grams/day ± sem) of chicks prior to Eimeria tenella challenge.

Day	Hen	No hen	P	Additive	No additive	P
0-3	2.53 ± 0.19	2.95 ± 0.18	0.11	2.87 ± 0.21	2.61 ± 0.15	0.32
3-5	3.74 ± 0.21	5.10 ± 0.21	<0.001	4.44 ± 0.24	4.39 ± 0.17	0.86
5-7	4.89 ± 0.26	5.80 ± 0.26	0.01	5.20 ± 0.30	5.50 ± 0.21	0.43
7-11	4.17 ± 0.17	6.51 ± 0.17	<0.001	5.64 ± 0.20	5.04 ± 0.14	0.01
11-15	3.66 ± 0.25	7.13 ± 0.25	<0.001	5.80 ± 0.29	4.99 ± 0.20	0.02
15-18	6.07 ± 0.45	7.50 ± 0.45	0.02	7.03 ± 0.52	6.54 ± 0.36	0.44

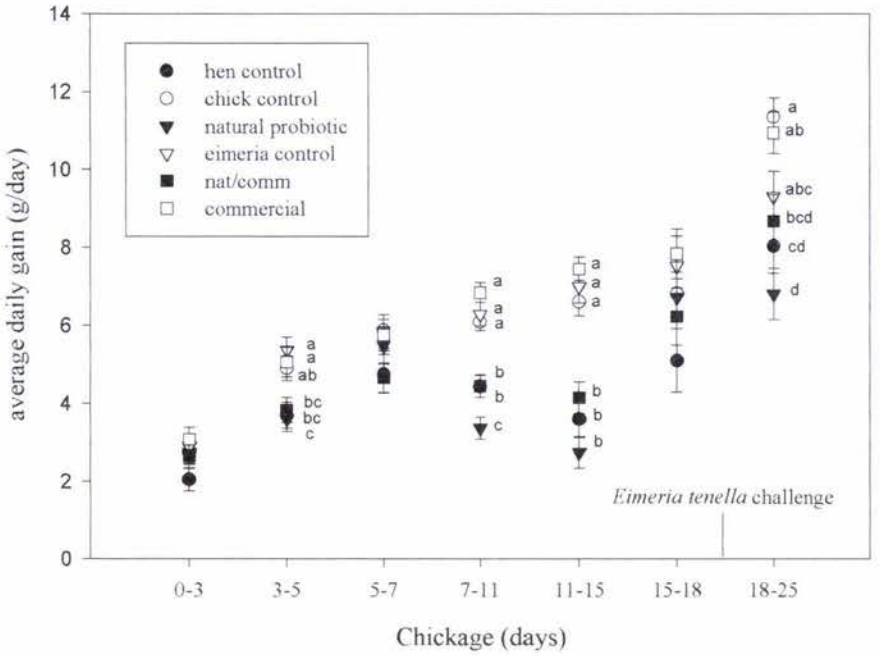

Figure 5.5 Effect of presence or absence of hens on chick average daily gain between weighing days.
Means without a common superscript are significantly different ($P<0.05$).

5.4.2 Behaviour: Open Field Test

5.4.2.1 Time to movement

On day 2, the no-hens group took longer to begin movement than the hens group (Table 5.7, Figure 5.6). This result was reversed on day 9 (however the differences were not significant) with the no-hens group showing shorter latencies to movement when placed in the open field than the hens group (39.8secs ±5.1 and 50.9secs ±5.1 respectively). By day 16, the original result from day 2 was repeated with the no-hens group again taking longer to begin movement than the hens group. The number of chicks that failed to move in the allowed time (2 minutes) followed the same pattern as above. On day 2, nine chicks from the hens group failed to move along with twelve from the no-hens group. This was followed on day 9 with nine again from the hens group, however in the no-hens group only 4 failed to move. On day 16 the number of chicks in the hens group that failed to move had

decreased to four, with eighteen chicks in the no-hens group remaining stationary. The effect of the commercial additive on time to movement was not significant.

Table 5.7 Effect of presence or absence of hens on chick behaviour in an open field: time to movement (seconds).
Means within a day without a common letter superscript are significantly different (P<0.05).

	Treatment (mean ± sem)		
Day	No-hens	Hens	P
2	59.9^{a}±5.1	44.2^{b}±5.1	<0.001
9	39.8 ±5.1	50.9 ±5.1	<0.001
16	65.9^{a} ±5.1	35.3^{b}±5.1	<0.001

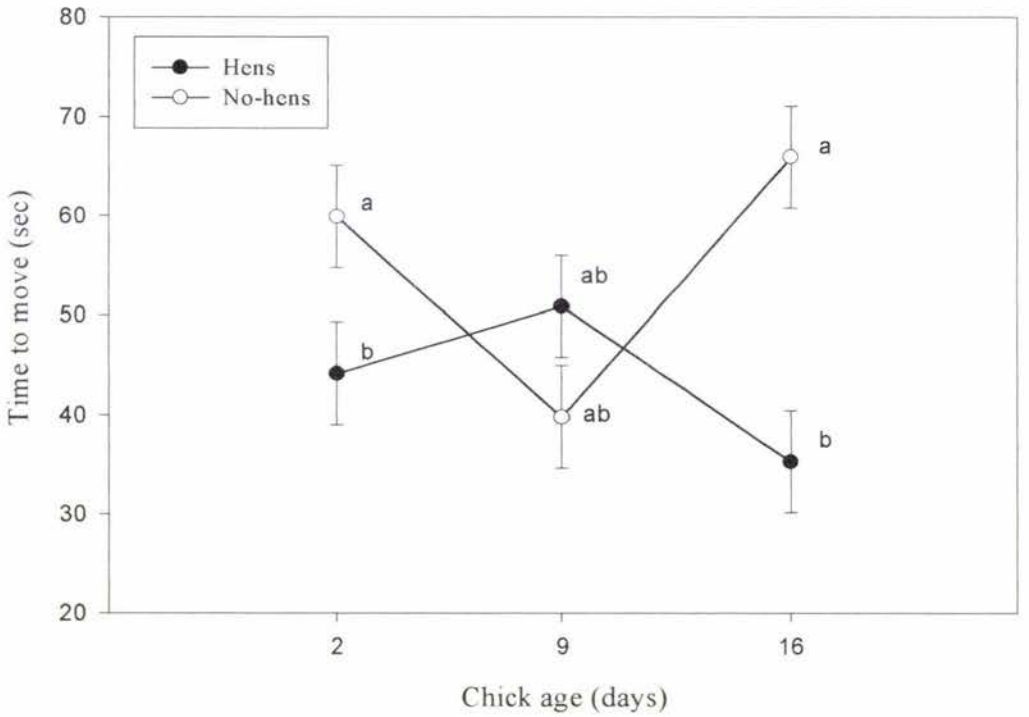

Figure 5.6 Effect of presence or absence of hens on chick behaviour in an open field: time to movement.
Means (± sem) within a day without a common letter are significantly different (P<0.05).

5.4.2.2 Time to vocalisation

The results for time to vocalisation in the open field followed a similar pattern as time to movement (Figures 5.6 and 5.7). On days 2 and 16 the no-hens group took longer to begin vocalisation when placed in the open field but on day 9 the hens took significantly more time (18.4 secs vs 10.4 secs, Table 5.8). All chicks vocalised on each of the days tested, and both treatment groups began vocalising before first movement. Again, the addition of the commercial additive had no effect on time to vocalisation.

Table 5.8 Effect of presence or absence of hens on chick behaviour in an open field: time to vocalisation (seconds).
Means within a day without a common superscript are significantly different ($P<0.05$).

	Treatment (mean ± sem)		
Day	No-hens	Hens	P
2	9.2^{a} ±2.0	2.4^{a} ±2.0	<0.001
9	10.4^{b} ±2.0	18.4^{a} ±2.0	<0.001
16	14.6^{a} ±2.0	6.7^{b} ±2.0	<0.001

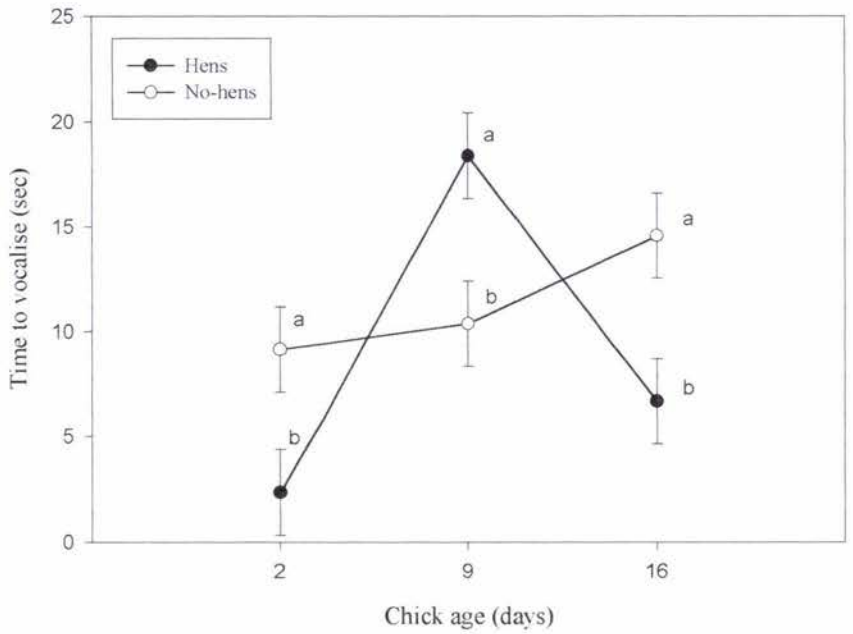

Figure 5.7 Effect of presence or absence of hens on chick behaviour in an open field: time to vocalisation.
Means (± sem) within a day without a common letter are significantly different ($P<0.05$).

5.4.3 Behaviour: Startle Response

5.4.3.1 Movement

There were no significant differences in the startle responses between chicks reared with hens and those reared without hens. The number of chicks responding to the startle with a defaecation, 'look away' and 'fly' response were too low to perform any statistical analysis on with the twelve chicks responding with flying on day 16 all in the hens group. One chick in the no-hens group responded by defaecating on the three testing days, although it was a different chick for each day. On day 2 and 9 chicks from the no-hens group only responded by looking away (2 and 1 chicks, respectively). On day 16 only one chick looked away when startled. This chick was from the hens group. Results of the other responses are shown on Table 5.9.

5.4.3.2 Vocalisation

The vocal responses to the startle response test were not significantly different for the two treatment groups on the three testing days (Table 5.9).

Table 5.9 Effect of presence or absence of hen on startle responses of chicks (30 per treatment) on 3 days prior to *Eimeria tenella* challenge.

	Hens			No Hens				
Day	2	9	16	2	9	16	χ^2	P
Freeze	3	9	1	5	20	3	0.21	0.90
Crouch	25	35	31	25	23	35	2.36	0.31
Jump	11	12	8	7	14	13	2.01	0.35
Run	21	4	7	20	1	8	1.75	0.42
Quiet	35	46	31	39	56	39	0.15	0.93
Peep	6	9	20	6	4	16	1.06	0.59
Loud peep	19	5	9	15	0	5	3.64	0.16

5.4.4 Behaviour: Scan

There were few significant differences in the behaviours for each treatment when observed in the home pen (Tables 5.10 and 5.11).

Table 5.10 Effect of presence or absence of hen and of day on chick behaviour in the home pen on 5 days prior to *Eimeria tenella* challenge in terms of percentage of chicks observed performing the behaviour.

Least-squares means, levels of significance, model R^2 values, and residual standard deviations (RSD) are given. Treatment-by-day interactions were not significant ($P > 0.05$). Means in the same row without common superscripts are significantly different ($P<0.05$).

	Treatment			Day of measurement							
	With-hen	No-hen	P	4	6	8	10	14	P	R^2	RSD
Eat	37.1	31.7	0.005	35.5	41.5	32.0	29.3	33.8	0.10ns	0.50	7.3
Drink	2.3	2.0	0.58ns	3.0	3.0	2.3	1.7	0.8	0.26ns	0.49	1.9
Sleep	21.3	24.8	0.55ns	24.3^{ab}	13.3^{a}	31.2^{b}	25.3^{ab}	21.7^{ab}	0.011	0.70	7.4
Walk	10.2	8.5	0.48ns	9.2	8.7	9.5	11.3	8.2	0.26ns	0.71	2.5
Stand	16.2	18.2	0.48ns	12.0^{a}	20.5^{ab}	13.0^{a}	17.5^{ab}	23.0^{b}	0.014	0.67	5.6
Sit	4.4	6.4	0.24ns	2.5	3.8	5.8	8.7	6.2	0.07ns	0.63	3.5
Other	9.4	9.1	0.95ns	14.5	9.5	8.0	7.0	7.3	0.20ns	0.61	5.8

Table 5.11 Effect of presence or absence of hens, presence or absence of commercial additive, or presence or absence of *Eimeria tenella* challenge on chick behaviour in the home pen on 2 days after *Eimeria tenella* challenge.

Data is percentages of each group observed performing the behaviour.

	Hens		No hens		Additive		No additive		*Eimeria*		No *Eimeria*	
Day	22	23	22	23	22	23	22	23	22	23	22	23
Eat	29	35	32	30	30	35	31	31	27.5	31	37	35
Drink	1	1	1	2	0.5	2	1	1	0.5	2.5	2	1
Sleep	15	21	28	24	17	18	24	25	27	23.5	11	22
Walk	10	9	7	6	10.5	8	7	8	9	6	7	10
Stand	18	15	13	12	12.5	15	17.5	13	14	14.5	19	12
Sit	12	13	11	16	14.5	15	9.75	14	13	14.5	8	14.5
Other	15	6	8	10	15	7	9.75	8	9	8	16	5.5

5.4.5 Eimeria tenella Challenge

Three chicks from the Commercial treatment group were found dead on day 24 and a further 4 were euthanased on day 24 because of severe sickness. These chicks were included in the results.

As seen in Figure 5.7 the hens control and no-hens control groups had lesion scores of zero. The effect on lesion score of presence or absence of hens was significant ($P<0.001$) with the hens group have a lower lesion score than the no-hens group. The hens and no-hens

groups that received the Eimeria challenge and the commercial additive had significantly higher lesion scores than the groups that only received the Eimeria challenge and the control groups (2.6, 1.8 and 0.0 respectively, $P<0.001$). The hen groups receiving the Eimeria challenge (± commercial additive) had significantly lower lesion scores than the corresponding no-hens groups ($P<0.001$). The data is grouped for ease of presentation.

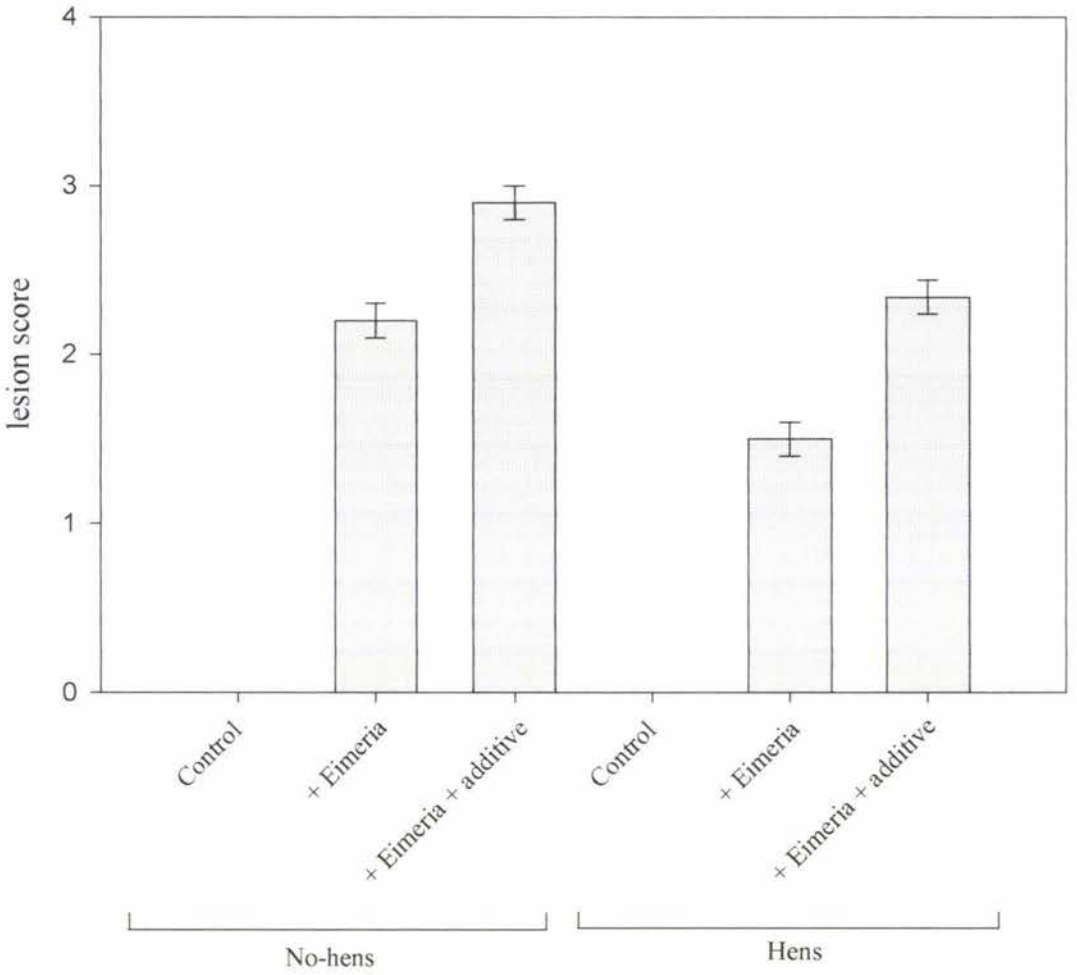

Figure 5.7 Lesion scores of chicks (± sem) after dosing with *Eimeria tenella*.
Scores were significantly lower for hens vs no-hens ($P<0.001$), control vs Eimeria ($P<0.001$) and Eimeria vs Eimeria + additive ($P<0.001$).

Figure 5.8 Chick with a caecal lesion score of 4.

Figure 5.9 Bleeding from the vent associated with *Eimeria tenella* infection.

5.5 DISCUSSION

5.5.1 Influence of hens on chick weight

Food recognition by the chick is complex (Kilgour and Dalton, 1984). Young birds peck at small particles and shiny surfaces and gradually learn to distinguish food and water (Appleby *et al.*, 1992). Under natural conditions, chicks' attention is directed towards food because they follow their mother around and, whenever she stops to peck at a food item, they gather round and join in the pecking activity (Appleby *et al.*, 1992). Social feeding is important in birds and results in greater feed intake per bird (Kilgour and Dalton, 1984). Feeding is, in some respects, a social activity and even when chicks are reared under commercial conditions they tend to feed as a group whenever possible (Appleby *et al.*, 1992).

The differences in treatment group weight in the present trial could have been due to the hens consuming most of the food leaving little for the chicks. However the scan behaviour for each treatment did not show any significant differences in the percentage of chicks observed eating in either treatment on the days observed ($P=0.721$). This result differed from Roden and Wechsler (1998) who found that the time spent feeding was significantly increased in chicks raised by a hen compared to chicks housed without a hen. They also found that number of chicks feeding at a given moment was significantly increased if the hen was feeding (Roden and Wechsler, 1998).

The average daily gains showed significant differences between the treatment groups containing chicks only and hens and chicks from days 7 to 15 ($P<0.05$). This was the time when the chicks reared with hens' growth rate lagged behind the chicks reared by themselves affecting the differences in weight. The Hen control, Natural probiotic and Nat/Comm treatment groups average daily gain's all decreased over this time period whereas the Chick control, Eimeria control and Commercial increased (Figure 5.5).

The difference in average daily gain between the treatment groups coincided with increases in the time to movement and vocalisation for the hens group and decreases in the time to movement and vocalisation for the no-hens group in the open field test.

5.5.2 Influence of hens on chick behaviour

Inactivity following placement in a strange environment has been reported to be an indicator of emotionality in the chicken (Candland and Nagy, 1969). Montevecchi *et al.* (1973) reported that such behaviour is a characteristic response of the chick to placement in a novel environment and that this response wanes with experience in that situation. Ginsberg *et al.* (1974) found relatively more fearful chicks took longer to spontaneously terminate immobility reaction, to terminate freezing behaviour in the open field, were less mobile in the open field and exhibited significantly fewer distress vocalisations both during freezing and the subsequent period of mobility in the open field. Jones and Merry (1988) examined the behavioural and adrenocortical responses of chicks exposed individually or in pairs to an open field and found there were no differences between treatment groups in either the durations of freezing or the latencies to vocalise. The authors' results where consistent with an interpretation of chicks' open-field responses based on the fear hypothesis.

Gallup and Suarez (1980), proposed that the behaviour of young chickens during their initial exposure to an open field can best be understood in terms of a conflict between attempts to minimise detectability in the face of potential predation. The authors found, contrary to the emotionality hypothesis, that birds maintained in social isolation for two days prior to testing were less likely than those kept in group-cages to move about or vocalise. In young chicks responses associated with attempts to reinstate social contact involve distress calling, searching behaviours, and attempts to escape from the confinement of the open field (Gallup and Suarez, 1980).

Andrew and Brennan (1983) found an increasing intensity of fear response in chicks from very low levels on day 1 to an early peak on day 2 or 3 followed by a trough, a larger peak

on days 5 and 7, a trough on day 8, and a final even larger rise with sustained high values from day 10. In the present study, on day 2, the no-hens group took significantly longer than the hens group to begin movement in the open field ($P<0.05$). This pattern was repeated on day 16, however on day 9 the trend was reversed with the hens group taking longer to begin moving in the open field. The difference on day 9 was not significant at $P<0.05$, although the mean difference between the treatment groups was noticeable (11 seconds).

The time to vocalisation followed the same trend. Time to vocalisation in the no-hens group gradually increased over the three testing days, whereas the hens group's response time increased markedly on day 9 before dropping again on day 16. The differences between the treatment groups were significant on all three testing days.

These results suggest that the chicks reared without hens were either more fearful or had less desire to reinstate social contact with cagemates on days 2 and 16, than chicks raised with hens. Roden and Wechsler (1998) found several factors indicating that chicks housed without a hen may be more fearful than chicks raised by a hen. The authors found that chicks reared without a hen showed significantly more flight responses in reaction to the standing up of the observer at the end of the observation period. It was also found that chicks reared without hens spent significantly more time standing which may indicate that vigilance was increased in these birds (Roden and Wechsler, 1998). The results of days 2 and 16, in the present study, were reversed on day 9 with the chicks raised with hens having increased fearfulness and less desire to reinstate social contact. On day 9 the chicks reared with hens took longer to begin vocalisation, but once started, exhibited piercing distress calls leading to the conclusion that they were signalling to the hens with a desire to reinstate social contact. Together with the decreased average daily gains, the results suggest that the chicks raised with the hens were more fearful and ate less in the in the second week of the experiment when compared to the chicks reared without hens.

In the present study all of the chicks tested in the open field exhibited vocalisation before movement. Gallup and Suarez (1980) also found that there were significant correlations

ranging from 0.58 to 0.95, between distress calls and ambulation latencies, with birds almost invariably emitting distress calls prior to moving about in the open field.

A startle response test can be performed immediately after the open field test as a complement (Lowndes and Davies, 1996). The results of our startle response tests showed no significant differences in reactions, either vocal or movement, between the two treatment groups. On day 2 the majority of chicks responded to the startle by crouching. This reaction was repeated over the following two testing days. The number of chicks responding to the startle by freezing was low on day 2 but increased markedly on day 9 and dropped again on day 16. This trend was the opposite when looking at the number of chicks responding by running. A large number chicks responded by running on day 2 followed by a large decrease on day 9 and a small increase again on day 16. On day 16, twelve chicks from the hens group reacted to the startle by flying.

Similar results were reported by Lowndes and Davies (1996) who found that upon exposure to an open field, chicks typically displayed an initial phase of quiet inactivity (including freezing) of variable length which was terminated by the onset of peeping, followed by movement. When they tested chicks from three different treatments (bilateral lesions to the archistriatum, bilateral electrode penetrations of the archistriatum without lesions or bilateral lateral cerebral area lesions) in an open field followed immediately by a startle response test, some responses to the open field test were significantly different. However, responses to the startle response test were not significantly different.

Scan behaviour in the home pens in the present study did not show significant differences between the treatment groups. These results are similar to a study by Roden and Wechsler (1998) who compared the behaviour of domestic chicks reared with and without a hen in an enriched pen. The authors found significant differences in standing and feeding behaviour between treatments, however there were no differences in any of the other behaviours observed.

5.5.3 Lesion Scores

Caecal coccidiosis is a parasitic disease in chickens, which is dependent upon the presence of microflora for maximum pathogenicity (Bradley and Radhakrishnan, 1973). Johansson and Searles (1948) studied changes in caecal flora of 2-week-old chicks during the course of infection with *Eimeria tenella*. They reported that the numbers of lactic acid bacteria and enterococci were reduced considerably by the 4th or 5th day of infections and numbers of coliform bacteria were unchanged in the caecum. Kimura *et al.* (1976) found that lactobacilli and bifidobacteria showed a remarkable decrease in number on the 5th day after infection with *E. tenella*.

The severity of caecal coccidiosis syndrome differed between chickens monoassociated with *Clostridium perfringens* and chickens monoassociated with *Lactobacillus* sp. (Bradley and Radhakrishnan, 1972). The authors found that the pathologic manifestations were more severe in the former than in the latter. Lesions were more severe in chickens monoassociated with *Streptococcus fecalis*, *Escherichia coli*, or *Bacteroides* sp. than in chickens monoassociated with *Lactobacillus* sp. when infected with standard doses of *E. tenella* oocysts (Bradley and Radhakrishnan, 1972).

In the present study, the addition of the synbiotic (Acid Pak 4-Way®) containing lactic acid bacteria to the water of the Commercial and Nat/Comm treatment groups did not decrease their infection with *Eimeria tenella* as could have been expected. Tortuero (1973) reported that constant feeding of lactobacilli reduced the severity of clinical disease of *E. tenella* infection.

Researchers have discovered that bacterial pathogens, such as *Escherichia coli*, salmonella and clostridia will attach to mannanoligosaccharides (MOS) (Cole and Close, 1999). MOS are complex, non-digestible carbohydrates contained in yeast cell walls (Cole and Close, 1999). If the pathogen attaches to the MOS, it loses the ability to infect the intestinal cell and is excreted from the body in the faeces (Cole and Close, 1999). The animal does not suffer any digestive disturbance or infection. Chicks receiving dietary MOS and orally

challenged with *Salmonella typhimurium* 29E had reduced concentrations of *S. typhimurium* 29E at day 10 (Spring *et al.*, 2000). This result was repeated using *Salmonella dublin*. Spring *et al.* (2000) found that MOS did not significantly reduce the concentrations of caecal coliforms although they were numerically lower. The authors found that it had no effect on caecal concentrations of lactobacilli, enterococci, anaerobic bacteria, lactate, volatile fatty acid, or caecal pH.

If the addition of MOS to the diet of the chicks did inhibit binding of certain pathogenic bacteria to the lining of the digestive tract of chicks without affecting the concentration of lactobacilli in the caeca, it could have been expected that along with the addition of lactic acid bacteria to the chicks, the infection of *Eimeria tenella* would have been limited. With the addition of an organic acid in the Acid Pak 4-Way® to the diet of the chicks also inhibiting the growth of pathogenic bacteria in the digestive tract, the lesion score results of the present study, showing that the commercial prebiotic and synbiotic increased *Eimeria tenella* infection, were unexpected.

In natural conditions, the newly hatched chick is quickly inoculated with intestinal micro-organisms from such sources as parental contact and adult faeces on litter and food. An important consideration is the present practice in the modern poultry industry of rearing chickens away from adult birds and in hygienic conditions where there is little opportunity for the chicken to acquire an indigenous intestinal flora (Lloyd *et al.*, 1977). Modern hygienic practices in poultry sheds, while admirable for control of many poultry pathogens, may result in a delay in the development of a stable indigenous flora of the intestine (Nurmi and Rantala, 1973; Lloyd *et al.*, 1977). This lack of indigenous flora could deny the host part of its natural defence mechanism against opportunistic enteric pathogens.

Oral administration of intestinal flora (faecal or caecal preparations) from adult birds into newly hatched chicks can diminish infection of poultry by *Salmonella* (Nurmi and Rantala, 1973). It has been suggested that native gut flora and *Salmonella* compete for the site of attachment on the intestinal wall of young chicks and protection is achieved by competitive exclusion of *Salmonella* (Stavric *et al.*, 1985). Impey *et al.* (1982) reported that the

colonisation of the caeca of newly hatched chicks by *Salmonella typhimurium* was prevented by oral administration of a mixture of cultures comprising 48 different bacterial strains originating from an adult bird know to be free of salmonellaes. In the present study the presence of the hens imparted partial resistance to coccidiosis caused by *Eimeria tenella* infection as shown by lower lesion scores (Figure 5.7).

Weight gain is an important method of assessing the severity of caecal coccidiosis (Visco and Burns, 1972). The phenomenon of weight gain in bacteria free, infected birds compared to weight loss in conventional and certain monofloral, infected birds suggests that certain species of bacteria interact with *E. tenella* in causing weight loss (Dykstra and Reid, 1977). Visco and Burns (1972) found the growth of the *E. tenella*-infected bacteria-free chicks was not as severely depressed as that of infected conventional chicks and indicated that the presence of the intestinal flora was involved in producing the disease syndrome.

In the present study, the average daily gain of the control and Eimeria + additive groups was significantly higher than the Eimeria group. If weight gain was used as a method for detecting coccidiosis in chickens, then the commercial prebiotic and synbiotic effectively masked the expected symptoms of the disease.

An interesting observation regarding the Commercial treatment group was that on day 5-post infection they seemed the healthiest animals of all of the treatments (excluding Hen control and Chick control). This was not the case, however, with three chicks found dead within 45 minutes of being observed behaving in what could best be described as normal when compared to pre infection with *Eimeria tenella*. A further 4 chicks in this group were euthanased because of sudden onset of severe sickness behaviour.

5.6 CONCLUSION

Chicks reared with hens appeared to be less stressed, as exhibited by a shorter freezing response to the open field test and a shorter delay to vocalisation in the first and third week of testing, compared to chicks reared without hens. The trend was reversed during the

second week with the chicks reared with hens being more stressed. This time of greater apparent stress was within a period when they grew significantly more slowly than the no-hen group. This led to the chicks reared without hens being heavier at 25 days. There were no significant effects of hens on spontaneous behaviour and startle responses of the chicks.

The combination of commercial prebiotic (Bio-Mos®) and symbiotic (Acid-Pak-4-Way®) caused increased *Eimeria tenella* infection with the presence of hens imparting partial resistance in chicks, but these effects were not reflected in differences in chick growth rates or weights, which were generally higher for the group receiving the commercial additive and for the group without hens.

Chapter 6

General Discussion

Food safety is top priority among consumers. Food is thought to play a major role in the transmission of microorganisms causing infectious diseases (Notermans, 1999) and food-borne illnesses are among the most widespread diseases of the contemporary world. Developments such as increasing world food trade, new production and processing technologies, increasing mass catering and changing eating habits make food-borne disease an evolving public health challenge (Notermans, 1999).

The control of food-borne diseases depends on understanding their mechanism of transmission well enough to prevent it (Notermans, 1999). A knowledge of factors contributing to food-borne disease facilitates the identification of specific control measures, and hence the prevention of such disease (Notermans, 1999). It is important that we attempt to control the carriage of potential food poisoning organisms in the live animal and minimise the opportunities for contamination of carcasses in the abattoir or processing plant (Mackey, 1989). Knowledge of the characteristics of food-borne organisms enables the identification of suitable conditions for food processing and preparation and if these conditions are well-selected, the growth of microorganisms can be inhibited or the organisms can be destroyed (Notermans, 1999).

Food products can become contaminated during various stages of production (Notermans, 1999). Preventing humans from suffering the effects of food contamination clearly starts at the agricultural stage (Byrne, 1998). Food manufacturers need to be assured that the raw materials they use are wholesome (Byrne, 1998). Many agents of foodborne disease are carried by red meat animals and poultry but there are wide differences between and within species in the incidence of affected animals and numbers of bacteria present (Mackey, 1989).

The chain of events involved in primary production, harvesting, processing, distribution and final preparation is quite long, and there are many opportunities for the food to become

contaminated (Notermans, 1999). Most organisms causing food poisoning must grow in food before there are sufficient numbers or sufficient amount of toxin to cause symptoms (Hobbs, 1974). It is important to attempt to minimise the carriage of pathogens in live animals and the contamination of carcasses in the processing plant. A variety of methods have been developed to reduce the levels of contaminating bacteria on carcasses. Most of the current methods focus on washing and sanitising procedures, but contamination can be also minimised by good manufacturing processes and by attention to general sanitation and hygiene in the processing plant.

6.1 PRESLAUGHTER FEEDING OF CATTLE

The possibility of reducing the number of potential meat-borne pathogens entering the meat processing plant through the manipulation of diet in the preslaughter or growing period was investigated in the studies reported herein. At the same time it was important to ensure that there were no adverse effects on production (i.e. carcass weight loss) and animal welfare.

Previous studies have indicated that fasting animals prior to slaughter can increase ruminal and faecal *E. coli* counts. Brownlie & Grau (1967) and Grau *et al.* (1969) found fasting increased *E. coli* in the rumen of sheep. Jordan & McEwen (1998) determined that fasting for 48 hours increased *E. coli* in the faeces of cattle by 1.4-2.4 log counts g^{-1}, depending on the preceding diet (feedlot ration versus high-roughage ration).

The initial study (Chapter 3) involving the effect of four preslaughter diets (48 hr hay, 24 hr hay, 24 hr fast and pasture) in steers found that fasting animals for 42 hours (including transport and overnight lairage) before for slaughter, substantially increased both the concentration and total numbers of *E. coli* within the digestive system, in comparison with animals fed before transport.

It was concluded from this trial that the preslaughter feeding treatment which offered the most advantages was the provision of hay for 48 hours before despatch. This method helped to reduce the gut burden and excretion of *E. coli*.

There are a number of possible explanations for the suppression of *E. coli* in the 48-hour hay group. These include the effect of coumarins, the 'broom' effect where bacteria attach to increased dietary fibre and are swept out of the gastrointestinal tract, the effect of antimicrobials produced by fungi on hay, the introduction of hay-borne bacteria acting as an inoculant of competitive bacteria and the alteration of dietary substrates affecting the population dynamics to the advantage of *E. coli* competitors.

The second cattle study (Chapter 4) expanded the preslaughter feeding regimes from four to eight. The feedstuffs were chosen for their differing coumarin content, dietary fibre composition and microbial composition. This study also demonstrated that fasting for 40 hours before slaughter (including transportation and overnight lairage) increased *E. coli* in the rumen and rectum at slaughter, while the feeding of some conserved forages reduced *E. coli* in both the rumen and rectum contents.

A number of mechanisms could have been acting to reduce *E. coli* in the rumen in this case. These include differing levels of VFA production from feeds after fermentation in the rumen, the presence of anti-microbial coumarins or other compounds in the feeds, the ingestion of different communities of microbes present in the feeds, and/or competition between *E. coli* and other rumen microflora.

Red clover hay and en-silaged pasture (haylage) were the most effective pre-transport diets for reducing *E. coli* counts in the rumen. This was most probably a consequence of increased production of *E. coli*-toxic volatile fatty acids, and additionally for haylage, the direct ingestion of lactic acid produced during fermentation in the haylage bale. The reduction of ruminal and faecal *E. coli* in lucerne hay-fed, and to a lesser degree, in meadow hay-fed heifers, may also be coumarin associated.

When cattle are fasted before transport, the weight of gut contents decreases but the material usually becomes more liquid (Bass and Duganzich 1980). In other words the dry matter percentage of the digesta decreases with progressive starvation. The initial study

confirmed this effect and produced the following new information. Firstly, if cattle are taken directly from pasture and transported to the processing plant where they are slaughtered 16 hours later, the total weight of gut contents and the total weight of water in the gut contents are similar to those seen in cattle which are fasted for 24 hours before transport. Secondly, even though cattle that are fasted usually have more watery digesta, the cattle that were pasture-fed up to the time of trucking had more runny faeces. In addition, the pasture-fed animals were dirtier, in terms of fresh faecal soiling on the hide.

The second trial involving eight preslaughter feeding regimes in heifers found that preslaughter dietary treatments had a highly significant effect on the dry matter percentage of the rumen contents at slaughter. Animals fed lucerne hay had drier rumen contents than haylage fed animals, while the dry matter percentage of the rumen contents of fasted animals was significantly lower than that of all other treatments. The dry matter percentage of faeces collected from the rectum after slaughter was not affected by the preslaughter dietary treatments or by the seasonal separation of the two trial replicates.

Dirtiness scores of animals' hides immediately prior to slaughter did not differ significantly between treatments in the numbers of animals with wet faeces present on the bung, brisket, butt, under the neck, or overall.

The manipulation of pre-slaughter cattle management can affect factors other than gut microbiology that are also of importance in a commercial operation. Pre-slaughter management can affect animal stress and dehydration, and thus welfare. Stress and dehydration can also detrimentally affect the production parameters carcass weight and meat quality (meat stickiness, pH and associated quality defects) (Gregory, 1998).

Dietary treatments investigated in both studies with cattle did not increase stress at slaughter, as shown by a lack of treatment effects on plasma cortisol, a commonly used indicator of stress (Gregory 1998). Meat ultimate pH, meat stickiness, and the incidence of blood splash were similar for all treatments.

Another benefit of feeding cattle before transport to slaughter as opposed to fasting may be the protection of carcass weight. During the initial trial preslaughter liveweight loss was comparable in the four treatments, and it is suspected that the differences in weight loss during transport were a reflection of differences in gut fill at the start of the journey. The dressing-out percentages for each treatment group increased when based on the final weight taken just before slaughter, due to higher levels of gut fill in the three treatments that were fed during the preslaughter period.

During the second trial involving heifers the mean carcass weight of fed heifers in the present study was 5kg heavier than that of the fasted heifers. The maintenance of carcass weight when cattle are fed hay or pasture silage, in comparison to fasting, before transport to slaughter has been reported in previous studies. For example, carcasses of Friesian bulls fed meadow hay or haylage for up to 20 h before transport for slaughter were slightly, but significantly, heavier than those fasted for the same length of time (Jacobson and Cook, 1997; Jacobson *et al.*, 1998). A simple cost-benefit assessment undertaken in one of these studies, in the economic climate of the time, showed an increased return of about $NZ 15-20 per bull after the cost of the feed was taken into account (Jacobson *et al.*, 1996). Further cost-benefit analyses of pre-transport feeding, taking into account carcass weight gains and reduced carcass contamination risks and also possible increased truck cleaning costs are needed.

6.2 ENVIRONMENTAL EFFECTS ON CHICKEN PERFORMANCE

Chicks reared with hens appeared to be less stressed, as exhibited by a shorter freezing response to the open field test and a shorter delay to vocalisation in the first and third week of testing, compared to chicks reared without hens, but the trend was reversed during the second week with the chicks reared with hens being more stressed. This time of greater apparent stress was within a period when the chicks with hens grew significantly more slowly than the no-hen group, leading to them being significantly heavier at 25 days. There were no significant effects of hens on spontaneous behaviour or the startle responses of the chicks.

The combination of commercial prebiotic (Bio-Mos®) and symbiotic (Acid-Pak-4-Way®) in the diet of the chicks caused increased *Eimeria tenella* infection following a challenge, with the presence of hens imparting partial resistance in chicks. These effects were not reflected in differences in chick growth rates or weights, however, as these were generally higher for the group receiving the commercial additive and for the group without hens.

6.3 IMPLICATIONS FOR MEAT PRODUCTION AND AREAS FOR FURTHER RESEARCH

The manipulation of diets in the preslaughter and growing period have been shown here to significantly affect some aspects of gastrointestinal microbiology in cattle and chickens. The addition of hay to the preslaughter diet of cattle provides for a method of decreasing the concentration and total burden of *E. coli* in the gastrointestinal tract, which in turn could lead to improved food safety. Surface soiling of hides was also decreased in hay-fed animals. This, along with an increase in dry matter percentage and decrease in the runniness of faeces, indicates potential for reduction of contamination of carcasses entering the abattoir and in the case of spillage in the abattoir. The reduction in contamination and surface soiling should lead to improved line efficiencies and decreased need for trimming and washing of carcasses in the abattoir. These changes could lead to increased profitability for abattoirs.

The manipulation of preslaughter diet of cattle did not detrimentally effect other factors considered important in a commercial operation such as weight loss or gain. The effect of the preslaughter diets on carcass contamination with meat borne pathogens is an area of further study that could be investigated.

Control of diseases in animals destined for human consumption is important for animal welfare in addition to its importance for food safety and profitability. Meat consumers have shown an increasing level of concern about the welfare implications of animal production systems over recent years. For ethical reasons alone, production animals should have as

high a quality of life as possible, and certainly any treatment that may cause suffering is unacceptable.

The commercial additives used here in the diet of the chicks during their growing period led to an increased infection following an *Eimeria tenella* challenge, with the presence of hens imparting partial resistance. An area of further investigation would be to investigate the reasons for this unexpected effect of the additives.

Animals that are diseased very often have difficulty coping with their environment, or fail to do so; hence their welfare is poorer than that of a healthy animal in otherwise comparable conditions (Fraser and Broom, 1990). Reductions in weight gain is a common method for detecting *Eimeria tenella* infection in chickens. The commercial additives in the diet of chicks effectively masked this symptom of the disease in the current study leading to non-detection of the severity of infection until dissection. These chicks also seemed to be the 'healthiest' of the groups of chicks, showing increased movement around the pen when compared to other infected groups. Seeking an explanation for these apparently contradictory responses could be an area of further study.

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