Inflammatory response to diet in the hindgut of layers

A report for the Australian Egg Corporation Pty Ltd

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Foreword

In a previous project, Taylor (2002) demonstrated that a fermentative or lactic acidosis could be incurred in the hindgut of layer stock exposed to a novel cereal-based feed. This work also suggested that a change in the proportion of a cereal type to which the bird had had experience could also lead to the condition. The development of the acidosis could occur in as little as 48 h. Lactic acid concentrations could increase to levels associated with acidosis in other animals such as ruminants and monogastric animals including humans.

The aim of the current project was to investigate the effects of diet change over the short and long term and under a wide range of conditions in various bird types such as post-peak layers, broilers and SPF Leghorns. In particular, the creation of a 'colitis-like' response of the hindgut mucosa to the dietary change was of interest as the presence of fresh blood and mucus, symptoms presenting upon irritation of gut tissues, was noted in growers, layers and broilers in earlier work.

The current work demonstrated that changing the cereal base of the diet given to both layers and broilers did produce an inflammation of the hindgut tissues and led to the loss of fresh blood and mucus within 48 – 72 h. In older layers, a change in the form of a wheat-based diet resulted in differences in blood loss over the productive life of the bird. The blood loss was associated with a depression in faecal excreta pH and increases in lactic acid concentrations. In layers, a greater response to the changed diet produced evidence of increased ileal inflammation as evidenced by histopathological examination. Broilers responded to different grains over a longer period of up to 16 d and the use of an antimicrobial did not prevent 'colitis-like' effects after a known "problem" grain, was fed to older broilers in the late starter period. In SPF Leghorns fed sterilised diets, changes in pH and organic acid concentrations occurred as in commercial birds. Additionally the application of cholera vaccine with a bacterial-based adjuvant, had effects on gut conditions in SPF birds.

A change in the cereal base of the diet, either in type or quantity, should be approached with caution for effects on fermentation conditions in the hindgut of poultry. The responses demonstrated in this work indicate that enteric disease may be precipitated by a simple change in the diet or may result in mild changes in the gut that, however, set the bird up for subsequent disease episodes.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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James Kellaway
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Executive Summary

As demonstrated in an earlier project, the hypothesis that poultry could incur a lactic or fermentative acidosis in the hindgut after a change in the cereal base of the diet was supported by changes in digesta and excreta pH and increases in the lactic acid fraction of the organic acids. Organic acids are the end product of microbial fermentation of substrates in the digesta, from endogenous losses of nutrients in many forms and from the breakdown of the microbiota themselves. Changes to the diet will change the conditions in the hindgut as the microbial populations adapt to different nutrient types and loads and how the animal, microbial and feed ingredient enzymes affect the process of digestion. It is in this adaptation phase that conditions may be created that have a negative effect on the birds' mucosal integrity. This, in the short term, may appear to be of little consequence, but has the potential to create conditions which lead to bouts of enteric disease at a later stage in the production cycle.

This project was designed to pursue the effects of cereal changes upon the hindgut tissues. In particular, the distal ileum was considered to be vulnerable to the effects of changes in lactic acid concentrations. As in the previous project, dietary cereals were changed, the digesta/excreta conditions were monitored and additional measurements were taken to glean further evidence of acidosis having an effect in the hindgut. Microbiological studies were conducted and histopathology undertaken by a commercial body. In addition, the effect of short term diet change on the mucosal fatty acid profile was investigated as well as possible systemic effects of inflammation of the gut tissues.

Several classes of bird were used in these trials. Mature post-peak layers were used for the main trials. Long term effects of a difference in feed processing were investigated by analysis of a data set produced from an Australian and an imported layer strain; birds with great disparity of feed intake despite similar body mass. Broilers were used to obtain comparative data with the use of "problem" grain in both layer and meat-bird types. Anti-microbials were tested for effects in a range of cereals without diet substitution to broilers or when older broilers were offered novel diets. As the microbial load on or in a batch of grain or a manufactured feed has been discussed for its influences on subsequent results in nutrition trials based upon cold-processed feeds, SPF Leghorns were used to test dietary changes when feeds could be sterilised. Another area that has been little considered is that despite much discussion of and research into oral delivery of vaccines, many injected vaccines rely on adjuvants to ensure maximal response and adjuvants may have a microbial base. Therefore another method of testing feeds was added through the use of vaccinated SPF Leghorns.

As in the previous work, feeds were formulated upon the use of a single-cereal. The feed ingredients and the cereals were commercial products available to, or used by, the poultry industry in eastern Australia throughout the course of the project in 2001-2003.

Methods of analysis were investigated in some detail and required much testing. Repeated Measures analysis of variance was further developed from that method used in the previous project, to account more accurately for both within-bird and between-bird variation over the course of each experiment. Importantly, a method of analysing binary data, positive or negative responses to particular tests, over time, was developed from work conducted with Dr R.D. Murison of the University of New England. This allowed for a large data set from layer work funded by the RIRDC Egg Program committee to be analysed. The data set was produced from laying hens over the productive laying phase, from 24 – 51 weeks of age, and which, to date, appeared too difficult to analyse. This work allowed for the scoring of blood loss in fresh excreta and, at times, diarrhoea, in the birds to be analysed sensibly over time. These methods have, in part, been shown acceptable for publication in refereed international journals and should be adopted for wider use within the Australian industry and poultry research projects.

Preliminary results from earlier work showed that blood loss in the excreta was associated with a change in the cereal base of the diet. The layer trials showed that with a greater drop in pH, however transient, and with increases in lactic acid, fresh blood loss in the excreta occurred. In trials with rice, this blood loss, associated with diarrhoea, was incurred in 48 – 72 h and the histopathological studies revealed that a greater degree of ileal tissue inflammation was produced. Microbiological work showed that some suggestion of shifts in microbial populations was incurred, across gross measures of anaerobes, aerobes

and lactic acid bacteria, in the short term. These studies warrant further investigation as replication was restricted due to the cost of such work.

Broiler trials revealed the changes to be consistent across bird types as a rice diet or “high” and “low” AME wheat diet produced different changes in pH and lactic acid over the starter period. When different grains, wheat, rice, sorghum and barley were used from day-old, differences were not significant overall. Virginiamycin changed the pattern of blood loss over the same period but was not influenced by the grain used. The application of a “problem” wheat, HP S1, produced gross changes in the digesta and blood losses and diarrhoea were not ameliorated by application of avilamycin.

SPF Leghorns allowed for the effects of dietary change to be tested without the effects of novel microbial loads being ingested and which may have interfered with changes in digesta conditions in the short term. These considerations have been noted as part of experimental work with feed enzymes that has been funded over many years. However, little is known about the effects of ingestion of successive microbial loads on commercial feeds over the productive life of the bird. Differences were noted across cereals in birds fed on sterilised feeds. Little work in this area has been done; expense being the major constraint. Currently, the use of oral vaccination and methods of maximising vaccine efficacy are the subject of research and conjecture. In the current trials, in a situation where vaccine testing was more rigorous than that required by the Australian regulatory organisation, the effect of microbial adjuvants on the bird were investigate for their influence on the digestive process in the hindgut. The results suggest that the type of microbial adjuvant, its source and function should be considered for effects on normal gut function. While it is reasonable to consider that oral:systemic immunological effects for disease control may appeal, consideration of systemic immunological : digestive function should be made as productivity may be compromised.

These trials were limited; constrained by labour requirement and cost. However, consistent and repeatable evidence was gathered to show that even minor changes in dietary cereal types or amounts could cause changes in digestive function in the hindgut, particularly in the distal ileum, that could create conditions that led to damage of the mucosa and which , while apparently minor, could produce damage that may allow for enteric disease to occur either immediately or at a time removed form the apparent cause. Much of what is considered in the current research climate is geared towards development and/or testing of commercial products to meet disease challenge or to substitute for current antibiotic application. These current results suggest that dietary changes, part of everyday commercial production could be responsible for some conditions occurring. The major conclusion of the work is that conservatism in use of least cost formulation, based around care in the use of alternative cereals, may either ameliorate or prevent the development of many enteric disease challenges.

1. Introduction

1.1 Background to proposal

The need to optimize feed efficiency, addressed by many forms of feed additives and medications, and to overcome problems due to the nature of the feeds themselves, is important in determining returns from egg production. A proposition that a hindgut acidosis, associated with high levels of dietary carbohydrate, may affect poultry as other species, has been shown to exist in some circumstances when the cereal base of a diet is changed. A symptom of this, diarrhoea, and a "colitis-like" blood and mucus loss was identified in birds after dietary change in as little as 24 h. In other animals, including humans, it has a chronic effect on the animals ability to utilise the diet efficiently and secondary effects on the immune response have long term negative effects.

There are other issues to be considered when an animal displays such symptoms and the welfare aspects of creating problems in gut function must be addressed. The egg industry has shown itself willing to adapt to change when evidence is presented to indicate problems with production methods.

Secondarily, environmental issues increasingly concern the egg industry due to pressure from the urban neighbours of poultry farms. The mucus losses and diarrhoea indicated by this condition contribute to wet excreta produced by the bird and it is suggested that these problems may in some cases be stimulated by dietary change.

1.2 Relevance and benefits

The AEIA estimated the gross value of production of the Australian egg industry at $300M in 1998. Feed costs contribute substantially to total production costs and maximising feed utilisation is part of the effort to improve production efficiency. Cereal grains such as barley and sorghum are becoming more expensive and, in some areas or Australia, compounders and poultry producers are forced to change to grain types such as wheat that have not been relied upon previously. The research outlined here was proposed to extend the results found in RIRDC (Egg Program) project UNC-12A. The review for that project was equally relevant to this application as it was noted that a response to factors including pH change, appears to stimulate blood and mucus loss within 24-48 h after changing the cereal base of the diet.

Practical methods of addressing the condition may identify how to lessen or prevent the problem occurring immediately upon, or after, cereal changes. Most commercial feed additive products are aimed at improving feed efficiency and/or altering gut status so that negative impacts on production are avoided. More importantly, the withdrawal of commonly used antibiotics will have a growing and serious impact on methods of production.

This project involves sustainability issues such as bird welfare, immune status and feed utilisation efficiency with consequent effects on production as well as excreta moisture and nutrient losses.

1.3 Review and interaction

1.3.1 Inflammation and diet

The final project report (RIRDC UNC-12A) of Taylor (2002) provided data supporting the hypothesis that a depression in pH in the hindgut of layers (which includes the distal ileum which functions in a similar way to the colon in other animals) is similar in effect to the acidosis that occurs in other animals exposed to a high carbohydrate diet. In particular, this is supposed to be due to the rapid fermentation of starch in the rumen and/or hindgut of ruminants placed on grain diets in a feedlot (Allison *et al*., 1975). Equally, the condition has been described in horses (Garner *et al*. 1975), pigs, dogs, rodents (Clayton, 1999) and humans (Cummings, 1981). Generally, the production of lactate in large quantities reduces gut lumen pH, and, concomitantly with death of large numbers of gram negative micro-organisms producing lipopolysaccharide endotoxin, an immune response is elicited in the hindgut mucosa. Poultry were

excluded from the list of animals in one review (Rowe, 1999) but the reasons were purely speculative; based on the assumption that birds had been selected for so many generations on high cereal diets. In several trials (RIRDC project UNC-12A) when a new diet, of similar specification but high single-cereal inclusion, was fed to birds that had been fed long term on a commercial blend feed, a drop in excreta pH occurred within 24 hr. Although pH returned to "normal" within 2-3 d generally, ruminant trials indicate that the rapid drop in pH is enough to do long term damage. This is sensible as gut micro-organism populations are extremely sensitive to changes in gut lumen conditions; particularly pH. It was noted that pH often increased above the original level when it rose and this has been noted in ruminants (Clayton, 1999) and in a mouse model of acidosis (Clayton and Buffinton, 2000). In layers, the pH drop could be as little as half a unit. In ruminants, 18-40 % of dietary starch entering the ileum (Owens *et al*., 1986) can cause a fall in faecal pH from 6.9 to 6.0 with symptoms of acidosis then accruing. Starch fermentation in the caecum can increase faecal N loss and diarrhoea (Ørskov, 1986). Gram positive lactate producing bacteria such as *Streptococcus bovis* and *Lactobacillus sp*. (Hungate *et al*., 1952) increase in the caecum (Ørskov *et al*., 1970) and pH may fall below 5.0 with a reduction in caecal motility and scouring occur as an indicator of acidosis as colonic water uptake is reduced due to the acid load (Lee, 1977).

Great emphasis has been placed on the importance of enzyme technology in feed efficiency but the mode of action of the major commercial enzymes is still not clearly understood and results are inconsistent. It may be that means are being sought to counter the negative effects of feeding technology which is geared to the needs of automation and not the physiology of the bird. Endosperm cell walls of cereals are rich in arabinoxylans and glucans which are polymers of xylose and glucose. These monogastrics are solubilised polymers increasing intestinal viscosity which in turn reduces rates of digestion and absorption. This is currently alleviated by inclusion of xylanases and glucanases (obtained from microbial cultures) to hydrolyse these polymers to the respective monomers (Spencer *et al*., 2000). The exogenous feed enzymes applied in the poultry industry are directed, largely, at breaking down specific NSP's in grains (xylanases applied to wheat; β-glucanases applied to barley) but anecdotal evidence from commercial poultry nutritionists highlight variations in responses (e.g. consistent positive response in wheat-based diets to xylanases provided by one company but poor response to those from another). Further, anecdotal evidence from grain growers/egg producers suggests that manipulation of cultivar, growing conditions and grain storage can bypass the need for exogenous enzyme use in barley-based diets for layers. It appears that the consideration of enzyme activity which allows access of amylases to the starch content of the cereal grain is only directed at the lack of endogenous animal xylanases/glucanases; the putative dearth of such enzymes produced by gut flora, but almost completely fails to note that the grain itself has enzyme systems that break down the structural support to allow the starch to be utilized for the energy requirements of getting the developing plant germinated and into sunlight. Pettersson and Åman (1989) highlighted that gizzard function, through feed grinding, and bacterial and/or endogenous enzymes are largely responsible for fibre degradation in the bird.

Other factors, and the complex interaction of bird, feed, disease and management, provide the final return to the grower in productive terms.

The intensive farming of laying birds extends back to Roman times and was described in detail by Pliny the Elder in his Natural Histories (Rackham, 1940). He noted that a laying hen could lay as many as 60 eggs on consecutive days, which suggests that highly productive types were selected. The naturalist/hunters of the 18-19th centuries provided detail of the natural feeds and foraging behaviour, details confirmed and extended by later studies (Baker, 1928; Beebe, 1931), of jungle fowl types; the progenitors of modern poultry. The birds had a fixed range, had a morning and evening feeding "circuit" and ate grains, green material and insects then spent the day and evening resting and slowly digesting the contents from a full crop. This surely provides a guide as to the development and ultimate function of the digestive tract of the bird. The free provision of a highly ground, often refined and cooked feed, renders the functional aspects of several gut organs almost redundant but their presence may induce untoward effects on digesta.

Argument as to the best method of feeding and managing laying hens was found in the late 1800's (Roland, 1986) and has continued to the present day. It has been noted that the mechanical needs of the production system have determined the way feed is processed and fed to the birds (Karunajeewa, 1978; Summers and Leeson, 1979); the requirements of the birds' gut being secondary.

Detailed experiments have re-emerged (particularly in the UK and Europe) with methods of grain processing, meal feeding and even "wet"-feeding being tested (Yalda and Forbes, 1995; Preston *et al*., 2000). Improvements in feed utilisation and lessening of disease problems (e.g. necrotic enteritis) have been found. In part, these methods recognise the functional and/or developmental aspects of the upper gut (crop, proventriculus and gizzard) which have been circumvented by feeding fine ground and cooked (though generally not to layers) feeds to poultry.

Additional broiler trials RIRDC (Egg Program) project UNC-12A

Three broiler trials were undertaken as, early in the project, laying birds were being raised from day-old and some techniques required testing e.g. pH measurement of excreta and caecal contents, development of the VFA method and column testing. It was deemed appropriate to add further results, by interactions of manipulating grain processing, grain types and enzyme use in a bird type that provided a "compressed" lifetime and had a large throughput of feed.

The results indicated that excreta pH was depressed after 24 h access to a new feed and was modified by grain processing and enzyme addition e.g. pH was lower in the excreta of broilers given a standard hammer-milled then pelleted wheat when enzyme was added rather than no enzyme addition and pH remained higher with whole grain included in the pellet. Development of proventricular dilatation was reduced in birds given whole grain. Further, there was a significant reduction in ascites mortality in birds given whole grain irrespective of cereal type (this included wheat, triticale and barley on a sorghum basal diet and a wheat on wheat basal diet). In these experiments, the use of whole grain in the pelleted food offered to the birds, generally depressed bird performance in the starter phase, with either a decrease in bird bodyweight or food efficiency. This depression in performance was offset as the birds aged or in one case by the use of exogenous food enzymes. It is likely that the decrease in performance in the starter phase when the birds were fed diets containing whole grain was due to the lack of development of the gastro-intestinal tract and a response to the novel form of the food. Initially, the mechanical grinding of the grain fraction prior to pelleting and which increases the surface area of the grain, allows for improved digestion. However, as the birds aged and the relative development of the gastro-intestinal tract increased, the birds offered the whole grain diet were better able to digest the food on offer, such that by 42 d of age, no differences in bodyweight were observed between the treatments imposed in both experiments. With a concomitant improvement in food conversion efficiency by the birds offered the whole grain during the grower phase, this indicates that the birds may have undergone a period of compensatory growth in the grower phase, after digestive tract adaptation had occurred. The adaptation of the digestive tract of the birds, facilitated by feeding diets containing large fibre particles is contrary to an assertion that the feeding of whole grain to broilers decreased food efficiency (Rowe *et al*., 1999). It is unlikely that the responses observed were related to differences in pellet quality. The addition of only 200 g/kg whole grain and the standard addition of 50 g/kg water to the diets as a binding agent resulted in similar pellet integrity between treatments. Most of the whole grain was crushed by the pelleting process which would have also aided pellet quality, although the crushed grains themselves were still readily distinguishable in the pellets. The lack of development of the gizzard, a result of feeding diets containing ground grain, may lead to the onset of proventricular hypertrophy and dilatation which may increase bird mortality due to ascites, through occlusion of the thoracic cavity, thereby impairing heart and lung function. The lack of gastro-intestinal tract development in the birds fed the ground grain diets is also evidenced by the birds' positive responses to dietary enzyme addition. The use of exogenous enzymes has been primarily to reduce the effects of non-starch polysaccharides which increase the viscosity of the digesta and limit nutrient absorption. However, the use of food enzymes may not produce consistent responses. The more muscular gizzard observed at 42 d when the whole grain diets were fed may allow greater intestinal and/or pancreatic reflux thereby improving digestion via both the birds' and the grains' natural enzyme activities as the feed stays in the upper digestive tract for longer periods (this occurs in layers; Taylor, 1998). That the bird is better able to 'handle' viscous diets with increasing age and that non-starch polysaccharides are not degraded by the birds' enzymatic systems, points to the role of the grains endogenous enzymes in the digestive processes. "Wet" feeding may lead to the early solubilisation of dry matter and crude protein, an outcome similar to when large dietary particles are retained in the crop, and suggesting the potential influence of a suite of enzymes not currently utilised.

Additionally, the common method of AME determination involves the use of whole grains, often in a "purified" diet with casein providing most of the protein base, which are generally cold pelleted. The birds are given three days adaptation to the test feed then a four day measurement period ensues. Given the above results it is suggested that adaptation takes at least 21 d. Given this result, positive response to exogenous enzymes from such tests may simply be due to the enzyme overcoming the depression in gut function during the early adaptation period.

1.3.2 Inflammatory bowel disease (IBD) and ulcerative colitis

As indicated above, these terms have been applied to the condition present in the birds after changes in diet but were due to the symptoms presenting, i.e. fresh blood and mucus with, generally, diarrhoea. The birds and excreta were inspected by an independent researcher in the acidosis/ulcerative colitis field in animals and the suggestion was that a "classical" condition was being expressed. It was noted with each succeeding trial and with development of observational techniques that blood and mucus was present after changes of diet. Generally it was necessary for the individual droppings to be broken apart for the full extent of blood and mucus to be appreciated. Ileal and colon samples were presented through a poultry industry veterinarian to NSW Agriculture for independent and anonymous histo-pathology analyses. Early results concluded that coccidiosis or enteritis were absent but that congestion of the superficial colorectal mucosa was present.

Inflammatory bowel disease (IBD) has two distinct clinical entities; ulcerative colitis (UC) and Crohn's Disease (CD) (Vernia *et al*., 1988b). UC affects the colon exclusively, but as indicated earlier (Taylor, 2002), the distal ileum in the bird has been described as functioning as the colon does in other animals, under the general term hindgut (Hill, 1983; Petersen et al., 1999). Vernia *et al*. (1988a) describe the IBD conditions as follows. Histopathologically, UC involves epithelial ulceration, loss of goblet cells, crypt abcesses and dense inflammatory infiltration of neutrophils, monocytes and macrophages, lymphocytes and plasma cells. CD causes inflammation anywhere in the gastro-intestinal tract but mainly in the distal ileum/proximal colon and involves discontinuous but full thickness lesions and granuloma is typical. IBD involves bleeding and diarrhoea and environmental influences include gut infections and the intestinal flora types present. The role of vaccines has been little mentioned but given the research into gut delivery of vaccines and the interactions with the animals immune system and IgA associated delivery of the response via Peyers Patches (reviews include those of Holzapfel *et al*., 1998; Dugas *et al*., 1999), it is to be considered that adverse effects in the gut may be stimulated by standard disease treatment (Of interest is the US promotion and use of bovine IgG in different animal types to stimulate and alter the gut environment of various classes of livestock including poultry. IgG treatment has been described in US research as profoundly altering weanling pig management. However, it is to be queried how the animals immune system responds after uptake of the foreign IgG through Peyers Patches and into the general circulation). In humans, dietary factors have not been implicated in causation of IBD but diet manipulation is effective in control of the active disease and cytokine studies indicate there is a delicate balance between microbial flora and the immune response or tolerance to the flora in the intestinal tract.

Many trials have been performed using animal models to mimic and examine inflammatory responses in the gut but a reproducible model is yet to be found (Kim and Berstad, 1992; Pacheco *et al*., 2000). Rodents have been used in many cases and criticised as a model for other animal types as they are coprophagic. However, chickens are coprophagic, with avid consumption of caecal contents occurring in many situations such as where low protein feeds have been used, and, with floor rearing, the birds can scarcely avoid consumption of excreta. Historically, competitive exclusion had been the base of some production systems and experimentation has been conducted into the use of various poultry gut homogenates in attempts to improve the productive efficiency of broiler birds. Lactic acidosis results in disease conditions in animals and there is evidence to suggest fibre fermentation in the human hind-gut, generally considered to be beneficial, is actually detrimental (Jacobs, 1989). A trial to link fermentation and hind-gut acid production with the onset of IBD, as colitis, was initiated with Dextran sulphate solution (DSS) in mice (Clayton and Buffinton, 2000). Faecal pH in the colitis group decreased over days 3-7. After 5 d diarrhoea, blood and mucus loss occurred in the colitis group then faecal pH rose significantly after. Faecal pH was not acutely acid but the subtle decrease contributed to the disease severity. DSS induced colitis was similar to grain induced lactic acidosis as diarrhoea and blood are seen in ruminant and human

faeces following hind-gut acid accumulation. The pH rise above the control group was possibly due to increased mucus secretion after intestinal damage as noted in sheep and TNF-α (a pro-inflammatory cytokine) levels increased. Similar models have been investigated with acetate induced colitis (the acetate is at levels seen in the human colon fermentation of large carbohydrate loads) and scoring methods used to note severity of inflammation (as ulceration, mucus cell depletion, crypt abscess, inflammatory cysts, mucosal atrophy, oedema, inflammatory cell infiltration and dilated vessels) (Sharon and Stenson, 1985; Kim and Berstad, 1992). Myeloperoxidase (MPO) activity in the colonic tissue can be measured as MPO is an enzyme present in neutrophils (lower levels in monocytes and macrophages) and is directly proportional to the neutrophil concentration in the inflamed tissue (Fabia *et al*., 1994). Morphological scores are highly correlated with MPO level. 4% acetic acid induced colitis can be maximal at 2 d with spontaneous healing by 12 d with graded ulceration and depth of injury in gut sections (Fedorak *et al*., 1990). Gross macroscopic changes are evident after 12 d but depth of injury is clear with histology with mucosal injury persistent at 4-6 d and lagging behind macroscopic improvement; similar in humans. Colonic secretion persists after 12 d so intestinal permeability and functional transport abnormalities persist despite normal appearance. Products of arachidonic acid metabolism, eicosanoids, mediate intestine inflammation and stimulate intestinal fluid and electrolyte secretions, possibly mediating the inflammatory response to IBD and contributing to the pathogenesis of diarrhoea (Sharon and Stenson, 1985). Human UC and CD sufferers have enhanced leukotriene and prostaglandin (PG) levels; both positively correlated with disease activity (Fedorak *et al*., 1990). The PG increase seen during the inflammatory phase may help protect the mucosa from insult.

Colonic epithelium in severe UC produces double the lactate of healthy mucosa and this may diffuse into the lumen (Roediger, 1989). Anaerobes exposed to an aerobic environment, through oxygenated blood loss into the colon lumen, ferment bacterial substrates to lactate and succinate rather than short chain fatty acids (SCFA = VFA). High levels of lactate have been reported in the rumen which can be affected by severe non-specific inflammation termed rumenitis. In fermentative organs, bacterially generated anions may vary with pH (below 5.5 SCFA production diminishes and lactate anions increase) and the severity of mucosal disease. SCFA (VFA) are C1-6 organic fatty acids, 85 % acetic, propionic and butyric acids in the nearly constant molar ratio 60:25:15 (Rombeau and Kripke, 1990). Carbohydrates reach the colon in 3 forms; NSP (dietary fibre/plant cell wall polysaccharides – cellulose, pectins, hemicellulose - resistant to the digestive enzymes in the upper tract); other polysaccharides, including resistant starch that resist digestion; simple carbohydrates that escape ileal absorption.

SCFA's are produced from pyruvate metabolism from glucose oxidation, with higher concentrations found with greater microbial populations (Rombeau and Kripke, 1982). The rumen and the human colon produce similar amounts from similar bacterial types. Caecal SCFA's in the rat are reduced 10-12 fold with removal of a high fibre diet but acetate and propionate are not changed as much as butyrate. SCFA absorption rates from the human, horse and pig colon are similar while ileal absorption, despite an efficiency like that of the colon, is associated with low concentrations. Ionised and non-ionised forms are absorbed from the lumen with transport being associated with bicarbonate ion accumulation. SCFA absorption stimulates Na absorption and may provide the energy source for its active transport. Intestinal problems like short bowel syndrome, small bowel atrophy and colitis are associated with SCFA perturbations. Net water transport is reduced and cell sloughing increases in rat ileum and colon with greater H^+ and lactate concentrations but with greater sensitivity of the ileal mucosa (Saunders and Sillery, 1982). High SCFA concentrations could be a factor in the diarrhoea found with cases of chronic carbohydrate malabsorption. Active UC in humans involves low faecal pH and, when quiescent, the pH is normal despite greater Na and Cl and lower K being found (Vernia *et al*., 1988a). Mild UC is associated with higher SCFA's while severe UC involves low SCFA's and high lactate levels and reduced pH. Some SCFA producing microbial populations are adversely affected by an acidic lumen while the lactate producers are stimulated. Intraluminal bleeding raises the oxygen concentration to create a favourable environment for facultative anaerobes including lactobacilli and streptococci.

IBD may be associated with increased lactate concentrations produced greater colonic carbohydrate levels, changes in microbial utilisation of the usual carbohydrate content or a reduction in the absorption of organic acids (Vernia *et al*., 1988b). In normal human subjects, higher colon lactate levels may result from more rapid transit of digesta interfering with conversion of ileal lactate to SCFA in the colon. In normal

humans and colitis sufferers, electrolyte and water absorption are stimulated by SCFA's but not so effectively by lactate.

Hampson *et al*., (2000) described the colitis in young pigs suffering mucohaemorrhagic diarrhoea produced by a colonic spirochaete, Serpulina hyodysenteriae. Dietary manipulation produced results contrary to expectation and highlight the negative effects of increasing hindgut fermentation with either dietary fibre or application of exogenous enzymes to increase ileal carbohydrate digestion.

1.4 Conclusion

Wolin (1981) stated that the microbial system in the rumen was well understood and highlighted the intense study of the dietary, microbial population and health interrelationships of the human intestine. Many of the intestinal diseases in the human are little understood due to these complex interactions. Despite the use of many animal models to attempt to reproduce the conditions, few provide consistent responses to allow for definite results to be produced. These same problems beset poultry production and are of much concern with the restriction of the availability of anti-microbial products. This project was designed to pursue the application of a dietary change to create fermentation changes in the hindgut of layer and broiler birds. A lactic acidosis appeared to be associated with the production of symptoms associated with a "colitis-like" effect within as little as 24 h. The effect was consistent across a range of cereal types and was studied to determine if an inflammatory response to diet change was present and under what conditions.

2. General Materials and Methods

2.1 Birds

Layer stock

The layer stock were a commercial, tinted-egg, layer cross (AZTEC x Lohmann Red) supplied by Bartter Enterprises (Griffith, N.S.W.). Chicks were hatched from fumigated eggs at the Bartter Enterprises Beresfield commercial hatchery. The chicks were vaccinated as per the current commercial programme at day old in the hatchery and thereafter on farm.

Housing

Insulated, tunnel ventilated, concrete-floored shed. Chicks were placed in Petersime electric brooders to 35 d thence floor-reared on hardwood litter. At 15 weeks, the growers were placed, and the feed trials were conducted, in modified Harrison carry-on cages.

2.2 Feeds

Commercial starter and grower diets

The birds were fed Weston Animal Nutrition starter crumbles from day old to 8 weeks, grower crumbles with coccidiostat to 17 weeks thence (control only) layer pellets for experiments 1-3. The experimental layer diets are described in Table 1.

Table 1: Experimental layer diets (g/kg).

Raw	Wheat (Commercial)	Wheat (HP S1)	Rice	Barley
Rice (80g/kg CP)			600.0	
Wheat (120 g/kg CP)	673.3			
Wheat (170 g/kg CP)		752.2		
Sorghum (90g/kg CP)				
Barley (100g/kg CP)				600.0
Soybean meal (475 g/kg CP)	107.0	43.0	134.0	135.0
Meat meal (520 g/kg CP)	97.0	80.0	130.0	108.0
Millrun (160 g/kg CP)	30.0	30.0	49.1	32.2
Sunflower oil	11.3	5.5	12.5	10.0
Tallow			6.5	39.5
Limestone	74.0	80.0	61.5	69.0
Lysine HCl	0.8	2.1		
DL-Methionine	1.6	1.5	1.7	1.65
Salt	2.5	2.5	2.5	2.5
Sodium bicarbonate		0.4		
Choline chloride	0.5	0.8	0.2	0.15
Vitamin/mineral layer premix[1]	2.0	2.0	2.0	2.0
Calculated specifications				
DM	898.3	904.2	888.2	902.6
Protein	182.7	200.3	180.3	179.9
fat EE	36.3		38.8	75.1
Linoleic	14.1	14.4	14.2	14.3
Ca	40.4		40.0	40.0
P	8.4		9.0	9.0
av. P	5.6		6.3	6.1
AME chick MJ/kg	11.50	11.51	11.52	11.52
Lysine	8.8		9.0	9.1
Methionine	4.2		4.6	4.2
Met + Cys	7.4		7.1	7.2
Threonine	6.0		6.1	6.4
Na	2.1		2.4	2.1
Cl	3.0		2.9	3.2

[1] The active ingredients (mg/kg) contained in the vitamin and mineral premix were as follows: retinol 1.2; cholecalciferol 0.075; all-*rac*-α-tocopherol acetate 5; menadione 0.46; riboflavin 2; pyridoxine HCl 1.78; biotin 5; niacin 10; vitamin B12 5; thiamine 0.25; D-calcium pantothenate 1.78; vitamin B4 choline 48; antioxidant 50; Mn 33; Fe 22.5; Cu 2.62; I 0.62; Se 0.05; Mo 0.2.

A commercial feed laboratory provided nitrogen analysis and starch analyses were provided by courtesy of a second laboratory (Table 2).

Table 2: Commercial laboratory analyses (duplicate samples) of experimental raws.

Raw	Protein (g kg^{-1} as is) laboratory 1	Starch (g kg^{-1} as is) laboratory 2	Starch (g kg^{-1} dry matter) laboratory 2
Wheat (Commercial blend)	143	576	627
Wheat (HP S1)	176	552	599
Barley	135	502	557
Rice	92	777	866
Soybean meal	474		
Meat meal	469		

The Graincorp HP S1 wheat, of 680 kg m^{-3}, had a 2 mm screen producing 15-25% of the sample, and was procured through Weston Animal Nutrition, Tamworth NSW.

2.3 Sample collections and measurements

In the introductory set of experiments (Chapter 3), birds of similar body weight were moved to individual trial cages and maintained on the commercial ration for 72 h prior to experimental treatments being imposed. In the second set of experiments (Chapter 4), the birds were placed in cages at 15 weeks of age and fed the commercial grower then layer rations until experimental diets were imposed for 72 h.

Methods were as described for Egg Program project UNC-12A. Prior to lights-on, excreta trays were scraped clean and fresh excreta pH was measured after dilution with 2-3 x deionised water (w/v). Samples were taken at 0 (feed change = control) and 12, 24, 36 and 48 h, plus 60 and 72 h where designated. Additional, excreta pH measurements were taken at 12 h intervals before the experiments as further "control" readings.

In addition to the excreta pH measures, fresh excreta were examined in detail and a score given to the presence or absence (1 or 0 respectively) of blood (usually in association with mucus) and diarrhoea. As with pH readings, such scoring was given at 12 h intervals prior to the trials as additional "control" readings.

To confirm the presence of blood in the faecal excreta, excreta were analysed using the Hemo FEC® faecal occult blood test (Boehringer Mannheim GmbH).

The birds were bled at 48 or 72 h and blood samples of approximately 3-4 ml were collected into EDTA tubes and placed immediately on ice for transfer to the laboratory for centrifugation (10 min at 3,000 x G). The plasma was transferred to a 2ml tube then frozen at –20° C. Immediately after bleeding the birds were euthanased by cervical dislocation and the hindgut was exposed for collection of digesta. For collection of samples for microbial counts, gloves were worn and washed with 70% ethanol as were instruments and the ileal and caecal surfaces wiped with tissues soaked in ethanol.

Digesta samples for microbiological counts were expressed directly into clean 5 ml syringes, the plunger replaced and depressed to exclude air, and which were placed immediately on ice. Samples were taken directly to a diagnostic laboratory (Bartter Enterprises) and following measurement of the volume (maximum sample size 1.0 ml) digesta were expressed into sterile tubes containing 9 ml of 0.1% peptone water. Samples were vortex mixed and tenfold serial dilutions were made. Total anaerobic and aerobic organism counts were made on Plate Count Agar (Oxoid CM 325) plates using a spiral plate method and inoculation of 50 µl per plate at 10^{-5} and 10^{-6} dilutions. Anaerobic plates were incubated in anaerobe jars with an anaerobic generating kit (Oxoid Anaerogen Kit ANO 25A) for 72 h at 30°C. Aerobic plates were incubated for 48 h at 30°C. Colonies were assessed and enumerated. Lactobacilli counts were determined from 10^{-6}, 10^{-7} and 10^{-8} dilutions with 1 ml samples inoculated on MRS agar (De Man, Rogosa, Sharpe; Oxoid CM 361) using a method to AS 1766.1.3 and with an overlay. Plates were incubated in anaerobe jars in microaerophilic conditions (Campygen; Oxoid CNO 25A) for 48 h at 37 °C. Presumptive Lactobacillus colonies were counted.

Following collection of digesta for microbiological counts, digesta from the jejunum, ileum, caeca and colon (gut section content collected where practicable) were collected into two tubes; samples for short-chain or volatile fatty acid (SCFA and VFA respectively) and lactic acid determinations. The tubes were immediately placed on ice for transport to the laboratory.

A section of distal ileum, taken from 3-6 cm above the ileo-caecal-colic junction, and colon, immediately below the junction, from randomly selected birds on each treatment, were sub-sampled and examined for gross pathology. Other sub-samples were subjected to commercial histopathology.

Preparation of digesta and plasma for VFA and lactic acid analyses was detailed in the previous report (Egg Program project UNC-12A; Taylor, 2002) but was standardised for the later experiments in the current project. A VFA standard diluent containing 4-methyl valeric acid at 200 ppm or de-ionised water were added to the digesta 3:1 w/w for VFA and lactic acid determinations respectively. Plasma samples were diluted 2:1. Digesta samples were then vortex mixed and centrifuged at 20,000 x G for 20 min (plasma samples 3,000 x G for 10 min). A 340 µL aliquot was added to a gas chromatograph (GC) vial insert then sealed and frozen.

VFA analyses were as described previously (Taylor, 2002). Data were initially analysed as concentration (ppm) and relative proportions of each VFA of the total volatile fatty acids (C1-7). Data were then converted to mmol/l for further analysis, which included $\log_{10}$ (x + 1) transformation of all data sets, and presentation.

L- and D-lactic acid concentrations (mMol) were as described previously (Taylor, 2002).

Plasma myeloperoxidase (MPO) concentration was measured using a Bioxytech (Oxis International Inc.) enzyme-linked immunosorbent assay (ELISA) kit. Absorbance values were read at 405 nm with final concentrations expressed in ng ml^{-1} according to a standard curve.

Preparation of the caecal slurry given to half of the layer birds (Chapter 4) was a simple process. Immediately prior to the dosing of the birds at day old, three 84 day-old SPF Leghorn cockerels were removed from a filtered-air, positive-pressure isolator and killed by cervical dislocation. The caeca were removed and the contents were expressed into a beaker, mixed and then vortex-mixed 10:1 (w/v) with phosphate-buffered saline (PBS) at 40ºC. Half of the birds were given an oral dose of 1 ml of the caecal slurry, the other half were dosed with 1 ml of PBS.

2.4 Statistical analyses

Scores of "colitis" and diarrhoea (binary data) were tested by comparing the change in deviance due to each treatment contrast with the critical region of the X^2_1 distribution, over time, using the GLM (family="binomial" model) of R (Ihaka and Gentleman, 1996).

Individual volatile fatty acid concentration and VFA proportion of total VFA, lactic acid data, digesta pH (where measured), microbiological counts (as CFU ml^{-1} and log transformed data) were analysed by analysis of variance using the GLM procedure of SAS (Release 6.12, SAS Institute Inc., Cary, USA).

Repeated measures data including feed intake and excreta pH were analysed using the PROC MIXED Model of SAS to model the covariance structure of the repeated measures and to account for changes of within-animal variance. Parameters expected to have possible pre- and post-treatment effects e.g. individual bird feed intake (d 1) and excreta pH (time = 0) etc. were initially included in the model as a covariate. Where a relationship was not found the parameter was not included in the final analysis.

Unless stated otherwise, data are presented as least square means (LS means ± standard error (SE)).

In tables, where data are presented as 0, it may be assumed that detection limits were not met for some measurements.

In some tables, data representing significant differences were presented in bold, to highlight them from a mass of data where no significant differences were found.

In later experimental chapters, while all data are presented in Tables, written description of results may include those for interactions e.g. between diet and sex. Tabulated results, for simplicity, describe main effects of diet and or sex (Tables 63, 64 and 72).

2.5 Ethical considerations

The experiments were approved by the Animal Care and Ethics Committee of Bartter Enterprises Pty Limited, under Animal Research Authority No's. 0004, 0031, 0125 and 0126 (acknowledged by the ACEC University of Newcastle). All work complied with the New South Wales Animal Research Act 1985 (as amended).

3. "Colitis-like" response and diarrhoea induced by dietary cereal change: introductory experiments.

3.1 Introduction

Little data provides an insight into the effect of a sudden change in the cereal base of a diet to layer stock; particularly to growers. Similarly, little evidence is available to indicate if such dietary change has an effect on conditions in the lower ileum. Previous work has generally concentrated on the caeca where fermentation is known to be active in poultry.

Wheat is a major component of poultry diets and has recently become predominantly so in some of the south-eastern areas of Australia that have previously relied on other cereals. This latter change in feed constituents has been driven by cost constraints. At times, wheat can be a problem in poultry feeds particularly with the use of new-season grain and, sporadically, because of adverse growing conditions, leading to changes in grain constituents and the production of "low AME" grain. These effects have been largely overcome with the recent commercial drive for exogenous enzyme (xylanase) inclusion into layer diets. In integrated operations, aging of wheat, and other cereals, has long been understood and practiced, largely through on-farm storage arrangements. With many producers in the layer industry buying feeds from commercial millers, the age, types and blends of grains are generally unknown and bird performance may be affected simply through a need for gut adaptation to a new feed. However, even in integrated operations, price advantages have historically lead to the cereal base of a diet being changed completely e.g. birds can be changed from a pure sorghum to a wheat-based diet.

A series of 48 h feeding trials over the grower and layer phases of a commercial, cross-bred, tinted-egg layer, with a range of commercial, single-cereal based diets were conducted to determine influences of feed change on excreta characteristics. In addition, a broiler trial conducted independently (Taylor and Jones, 2004a) was used to determine if blood might be detected in broiler excreta over the starter and grower phases when different grain processing was employed.

3.2 Materials and methods

These observations were added to several of the trials under-taken for the Rural Industries Research & Development Corporation (Egg Program) project UNC-12A when, after completion of a series of experiments, close observation of fresh faecal excreta from both broiler and layer stock revealed the presence of what appeared to be blood, usually associated with mucus, immediately after a dietary change.

As per General Materials and Methods described by Taylor (2002). Growers and layers of a commercial layer strain (Aztec 101 / 007 supplied by Bartter Enterprises Limited) and broilers (Ross 308, supplied by Bartter Enterprises Limited) were used to investigate and develop methods of scoring and confirming the presence of blood in the fresh faecal excreta of birds after feed changes were introduced. Binary data, denoting the presence or absence of blood, were collected at various periods of time after the excreta trays under the birds had been cleaned. Experienced staff of a poultry vaccine R&D, production and testing unit monitored the method and collection of data to ensure accuracy and consistency. An experienced poultry veterinarian provided an independent assessment of the methods employed to collect the data. Similarly, the application of the Hemo FEC® faecal occult blood test used to confirm blood scores was observed and monitored by both the vaccine test unit staff and the veterinarian. Diarrhoea was scored as 'present' when the faecal portion of individual excreta presented as wet and formless i.e. an amorphous mass. Detailed preliminary measurements indicated water contents of such excreta as being greater than the 75-80% range described as 'normal' (Larbier and Leclercq, 1992).

The method of analysis of the binary data was suggested and developed by Dr R.D. Murison (Division of Mathematics and Statistics, UNE) who contributed much time and effort to allow this project to proceed.

Broiler trial

Barley diet 200 g/kg whole or ground grain in a sorghum basal diet.

Grower trials

Wheat, Rice, Sorghum and Barley 13 weeks old.

Commercial (Ridley Agriproducts) and wheat-based grower diets; crumble, meal or wet-fed at 18 weeks old.

Layer trial

Wheat, Rice, Sorghum and Barley peak production.

Note: All 48 hour trials, within week specified.

3.3 Results

Broiler trial

Starter phase

A time x grain interaction (p = 0.031) involved the ground barley diet losing blood in the excreta earlier (z = 2.031, p = 0.042) than did the ground feed but with similar final numbers of birds losing blood in the excreta (Table 3). Enzyme application did not alter (p = 1.000) excreta blood loss.

Grower phase

Over time, a mean increase (p = 0.001) in blood loss in the excreta (Table 3), irrespective of grain processing or enzyme application, was found after the grower feed was presented to the birds at 21 d of age. A grain x enzyme interaction was suggested (p = 0.061).

Table 3. Probability estimates (± SE of the probability) of blood in the excreta of broilers in the starter and grower phases (n=8) fed a cold-pelleted, crumbled, sorghum-based diet incorporating 200 g/kg of either ground or whole barley.

Phase	Barley	Age (d)				
		8	11	14	15	18
Starter	Ground	0.11 0.136	0.17 0.095	0.26 0.067	0.38 0.068	0.51 0.095
	Whole	0.01 0.277	0.03 0.197	0.10 0.124	0.30 0.080	0.62 0.103
		25	30	32	35	40
Grower	Ground	0.48 0.093	0.56 0.064	0.63 0.054	0.70 0.068	0.76 0.094
	Whole	0.32 0.101	0.45 0.068	0.60 0.057	0.73 0.072	0.83 0.100

Grower trials

Wheat, Rice, Sorghum and Barley 13 weeks old.

Overall mean estimates of blood in the excreta (Table 4) indicated a difference between the cereals with rice producing a higher ($p = 0.022$) score than wheat, sorghum or barley. However, over time, a significant difference was suggested ($p = 0.057$). No differences ($p > 0.05$) between grains were found with diarrhoea scores (Table 4).

Table 4. Probability estimates (± SE of the probability) of diarrhoea or blood in the excreta of 13 week old growers (n=12) fed cold-pelleted, crumbled, wheat, rice, sorghum or barley-based diets for 48 h.

Factor	Cereal	Time (h)			
		12	24	36	48
Blood	Wheat	0.08 0.204	0.12 0.130	0.19 0.098	0.28 0.140
	Rice	0.24 0.137	0.27 0.088	0.31 0.083	0.34 0.125
	Sorghum	0.01 0.433	0.03 0.278	0.08 0.154	0.21 0.165
	Barley	0.05 0.268	0.06 0.173	0.07 0.156	0.08 0.234
		12	24	36	48
Diarrhoea	Wheat	–	0.08 0.233	0.08 0.147	0.08 0.233
	Rice	–	0.59 0.128	0.81 0.101	0.93 0.183
	Sorghum	–	0.19 0.165	0.19 0.104	0.19 0.165
	Barley	–	0.32 0.137	0.36 0.084	0.40 0.137

Commercial (Ridley Agriproducts) and wheat-based grower diets; crumble, meal or wet-fed at 18 weeks old.

More blood was lost ($p = 0.014$) when the birds were changed to the wheat feed but was not altered ($p > 0.05$) by the feeding method (Table 5). A feed x time interaction ($p < 0.01$) showed diarrhoea increasing rapidly over the last 12 h of the trial with wheat but, again, was unaffected ($p > 0.05$) by the feed method.

Table 5. Probability estimates (± SE of the probability) of diarrhoea or blood in the excreta of 18 week old growers (n=12) fed a commercial, crumbled diet or a cold-pelleted, crumbled, wheat-based diet for 48 h as either a crumble, meal or wet-fed.

Factor	Feed/Method	Time (h)			
		12	24	36	48
Blood	Commercial	0.01 0.383	0.01 0.245	0.02 0.178	0.02 0.248
	Wheat	0.01 0.244	0.03 0.156	0.07 0.091	0.16 0.105
	Crumble	0.01 0.330	0.02 0.211	0.05 0.133	0.09 0.167
	Meal	0.01 0.293	0.03 0.187	0.07 0.113	0.14 0.136
	Wet	<0.01 0.585	0.01 0.377	0.02 0.209	0.05 0.216

Table 5. (continued).

Factor	Feed/Method	Time (h)			
		12	24	36	48

Diarrhoea	Commercial	0.20 0.086	0.21 0.056	0.23 0.053	0.25 0.080
	Wheat	0.08 0.109	0.18 0.068	0.35 0.049	0.58 0.068
	Crumble	0.17 0.108	0.21 0.069	0.26 0.062	0.31 0.092
	Meal	0.23 0.096	0.32 0.061	0.42 0.055	0.53 0.081
	Wet	0.02 0.225	0.06 0.142	0.17 0.081	0.42 0.092

Layer trial

Wheat, Rice, Sorghum and Barley peak production 24 weeks old.

Both blood loss ($p < 0.001$) and diarrhoea ($p < 0.001$) (Table 6) increased over the experimental period. While excreta blood loss was not influenced ($p > 0.05$) by the different grains, there was a trend ($p = 0.070$) to more severe diarrhoea in birds on the rice diet than the other three grains.

Table 6. Probability estimates (± SE of the probability) of diarrhoea or blood in the excreta of 24 week old layers (n=12) at peak production fed cold-pelleted, crumbled, wheat, rice, sorghum or barley-based diets for 48 h.

Factor	Cereal	Time (h)					
		–24	–12	12	24	36	48
Blood	Wheat	0.06 0.183	0.09 0.139	0.14 0.101	0.20 0.078	0.29 0.084	0.39 0.113
	Rice	0.06 0.171	0.12 0.127	0.23 0.089	0.40 0.070	0.60 0.080	0.77 0.106
	Sorghum	0.05 0.202	0.08 0.154	0.11 0.112	0.16 0.089	0.22 0.097	0.29 0.129
	Barley	0.05 0.208	0.07 0.158	0.11 0.115	0.17 0.088	0.25 0.092	0.35 0.123
		–24	–12	12	24	36	48
Diarrhoea	Wheat	0.22 0.118	0.28 0.089	0.35 0.066	0.44 0.061	0.52 0.075	0.61 0.098
	Rice	0.41 0.114	0.62 0.077	0.80 0.077	0.90 0.107	0.96 0.148	0.98 0.192
	Sorghum	0.42 0.105	0.45 0.079	0.47 0.061	0.50 0.061	0.52 0.077	0.55 0.102
	Barley	0.40 0.107	0.46 0.079	0.52 0.061	0.58 0.061	0.63 0.078	0.69 0.102

3.4 Discussion

Some preliminary data collected from a range of trials with layer stock at various ages through the production cycle and broilers through the starter and grower phases was collected after observation of blood appearing in the excreta. In general this was associated with mucus loss and, at times, evidence of diarrhoea. The change in diet appeared to stimulate some blood loss from some birds within a short period; generally from 24 h after diet change. During the final stages of RIRDC (Egg Program) project UNC-12A, methods of dealing with these binary data, over time, were discussed with Dr R.D. Murison, statistician, University of New England. An appropriate analysis was suggested and developed for application to data from a series of experiments directed at establishing the wider responses to diet change in the hindgut of layers and broilers. In particular, the effect of diet change leading to pH reduction and increases in lactic acid in the distal ileum, caeca and colo-rectum (where possible) was studied for its association with fresh blood loss, diarrhoea and changes in the hindgut mucosae that could suggest that tissue damage had occurred.

In general, few if any observations were made of blood in the excreta of birds on their commercial rations. With further observation, after diet change, blood became apparent in as little as 24 h. Individual birds displayed blood loss, others did not. This led to the conclusion that little intermittent loss of blood occurred. Broiler trials were more complex as group cages provided for a "mean" observation that could have been based on one positive score amongst a mass of excreta. Broiler trials were simplified to score presence or absence of blood (or diarrhoea) in the first 2 or 4 hours after the excreta trays were cleaned. This was further refined in a final broiler trial (Chapter 6).

These consistent results provided the impetus for further work to investigate the potential hindgut tissue damage that may have been caused by dietary cereal changes.

4. "Colitis-like" response, diarrhoea and alterations to hindgut function after dietary cereal change to layers.

4.1 Introduction

The changes to excreta pH and organic acid concentrations found in previous work (Taylor, 2002) and the introductory experiments (see above) displayed some variation across the range of cereals employed and in layer stock of different ages. With the greater feed intake in laying hens, in the important period of peak production, some consistency in these results was noted. For the purposes of addressing the hypothesis that a dietary cereal change caused a hindgut lactic or fermentative acidosis, consistent increases in lactic acid concentrations, of both isomers, were found and were of such a magnitude as found in acidotic conditions in dogs (Clayton, 1999), humans (Vernia *et al*., 1988a) and sheep (Clayton, 1999; Ding and Xu, 2003).

Post-peak layers were tested when subjected to dietary change over a range of cereals to confirm the earlier (Taylor, 2002) findings and to pursue the effects of fermentation changes on the hindgut sections. The possibility of tissue damage was investigated using a range of methods.

4.2 Materials and methods

As per General Materials and Methods (above). Another group of birds (Lohmann Brown) were transferred from the floor to the test cages at 15 weeks. The birds were grown on commercial grower and layer diets until each experiment began. Three experiments were conducted;

1. Experiment one compared the commercial layer feed with two wheat-based diets. One diet was based on the commercial millers' wheat, the other on a known "problem" wheat designated HP S1 procured from Graincorp. Test feeds had been mixed with hammer-milled grain and then cold-pelleted. The experiment was split into three successive groups of 16 birds on the test diets. Each group had 16 birds, half undosed/dosed with caecal slurry (see Chapter 2 above) for each test diet for a total of 48 birds (16 per feed treatment).
2. Experiment two compared the commercial layer feed with a barley based feed (commercial feed grain, hammer-millled then cold-pelleted). Half the birds were undosed or dosed at day-old with the caecal slurry and then each group given the test feeds for a total of 16 birds (8 per feed treatment).
3. Experiment three was as per Experiment two but with rice as the test cereal.

4.3 Results

Experiment 1

Feed intake was not altered ($p > 0.05$) by changing the cereal base. Mean excreta pH (Table 7) changed over the trial period with a consistently lower ($p < 0.05$) pH in the morning than in the evening but the type of wheat did not alter ($P > 0.05$) excreta pH through the trial (Table 7).

A caecal dose x feed interaction was found with lower ($p < 0.05$) mean excreta pH from dosed birds on the HP S1 wheat than undosed birds (Table 8).

Digesta and plasma L- or D-lactic acid (Table 9) were unaltered ($p > 0.05$) by caecal dosing or feed change except for an increase ($p < 0.05$) in plasma L-lactate in birds dosed with caecal contents at day-old (5.66 and 7.02 ± 0.295 mMol in dosed and undosed birds respectively).

Table 7. Mean excreta pH and excreta pH of layers fed commercial or HP S1 wheat-based diets (n=8) for 72 h at 25/26 weeks.

Diet	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Mean pH	6.66 bc	7.45 a	6.16 e	6.99 b	6.20 e	6.73 bc	6.10 e	6.63 cd	6.27 de
SE					0.130				
Weston wheat	6.70	7.39	6.14	7.01	6.19	6.88	6.02	6.55	6.42
HP S1	6.62	7.51	6.18	6.97	6.21	6.58	6.17	6.71	6.12
SE					0.095				

Table 8. Excreta pH of layers fed commercial or HP S1 wheat-based diets (n=8) at 25/26 weeks and either dosed or undosed with caecal slurry.

Feed	Caecal dose	
	Undosed	Dosed
Weston commercial wheat	6.56 [ab]	6.62 [ab]
Graincorp HP S1	6.72 [a]	6.41 [b]
SE	0.086	

Table 9. Digesta and plasma concentration of L- and D-lactic acid (mMol/L) of layers fed commercial feed or commercial or HP S1 wheat-based diets (n=8) at 25/26 weeks.

Source	Feed	Lactic acid (mMol)			
		L-lactic acid		D-lactic acid	
		Undosed	Dosed	Undosed	Dosed
Jejunum	Commercial	29.36	26.40	14.68	12.64
	Weston wheat	26.47	31.26	11.22	14.46
	HP S1 wheat	29.08	41.75	7.33	10.23
	SE	6.534		5.073	
Ileum	Commercial	24.79	25.95	0	13.83
	Weston wheat	39.68	34.66	22.67	23.14
	HP S1 wheat	37.69	47.39	17.37	15.05
	SE	7.018		6.338	
Caeca	Commercial	2.95	3.43	4.62	4.66
	Weston wheat	2.56	0.75	2.96	1.20
	HP S1 wheat	0.97	1.04	1.65	1.89
	SE	1.027		1.231	
Plasma	Commercial	5.00	6.69	0.36	0.27
	Weston wheat	6.19	6.56	0.70	0.49
	HP S1 wheat	5.79	7.80	0.04	0.09
	SE	0.510		0.217	

Total aerobe and MRS (lactobacilli) counts did not differ ($p > 0.05$) across the ileum and caeca but total anaerobe counts were greater ($p < 0.05$) in the caeca than the ileum (Table 10). In the gut sections considered separately, ileal and caecal digesta microbial counts differed with the feed (Table 10) were not significantly altered ($p > 0.05$) by cereal type in the ileum or caeca.

No blood was noted in excreta before the test diets were fed to the birds. A time x feed interaction was found over the 72 hrs of the experiment with birds fed the HP S1 wheat losing more blood in their excreta with time (Table 11) compared with the birds continuing on the commercial diet ($z = 2.529$, $p = 0.011$). Birds on the Weston commercial wheat lost increasingly more blood over the trial but the probability estimates indicated that the mean scores were intermediate between the commercial and HP S1 diets. Excreta blood scores were not influenced ($p > 0.05$) by caecal dosing (Table 11).

Table 10. Ileal and caecal digesta microbial counts (cfu ml^{-1}) of layers fed commercial feed or commercial or HP S1 wheat-based diets (n=8) for 72 h at 25/26 weeks.

Source	Feed	Microbial counts (cfu x 10^9) per ml of digesta					
		Total anaerobes		Total aerobes		Lactobacilli	
		Undosed	Dosed	Undosed	Dosed	Undosed	Dosed
Ileum	Commercial	1.78 [b]	1.11 [b]	8.70 [a]	9.91 [a]	10.88	6.91
	Weston wheat	11.70 [a]	9.21 [a]	2.79 [b]	1.08 [b]	10.29	7.61
	HP S1 wheat	6.65 [a]	11.89 [a]	1.26 [b]	1.17 [b]	6.35	9.82
	SE	2.045 x 10^9		2.311 x 10^9		2.085 x 10^9	
Caeca	Commercial	15.51	9.68	17.28 [a]	12.73 [a]	21.04	12.44
	Weston wheat	13.15	36.73	1.24 [b]	2.14 [b]	9.60	12.76
	HP S1 wheat	20.78	32.02	4.15 [b]	3.02 [b]	11.55	11.15
	SE	1.083 x 10^{10}		2.493 x 10^9		3.410 x 10^9	

Table 11. Probability estimates of blood in the excreta (probability ± SE of the probability) of layers fed commercial feed or commercial or HP S1 wheat-based diets for 72 h at 25/26 weeks and birds undosed or dosed with caecal contents at day-old (n=8).

Feed	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Commercial	0.06 ± 0.158	0.06 ± 0.131	0.06 ± 0.108	0.06 ± 0.092	0.06 ± 0.086	0.06 ± 0.092	0.06 ± 0.108	0.06 ± 0.131	0.06 ± 0.158
Weston wheat	0.07 ± 0.136	0.08 ± 0.114	0.09 ± 0.094	0.11 ± 0.077	0.13 ± 0.066	0.15 ± 0.062	0.17 ± 0.069	0.20 ± 0.083	0.22 ± 0.101
HP S2 wheat	0.02 ± 0.173	0.03 ± 0.147	0.05 ± 0.121	0.08 ± 0.097	0.12 ± 0.077	0.17 ± 0.063	0.25 ± 0.060	0.34 ± 0.069	0.44 ± 0.086
Undosed	0.05 ± 0.123	0.06 ± 0.103	0.07 ± 0.085	0.09 ± 0.069	0.12 ± 0.057	0.15 ± 0.051	0.18 ± 0.054	0.22 ± 0.064	0.27 ± 0.078
Dosed	0.04 ± 0.129	0.05 ± 0.109	0.07 ± 0.090	0.08 ± 0.073	0.10 ± 0.061	0.12 ± 0.056	0.14 ± 0.060	0.17 ± 0.072	0.20 ± 0.088

A significant ($p = 0.03$) feed x dose effect (Table 12) indicated that birds continuing on the commercial feed but that had been dosed with caecal contents at day-old had lower ($p < 0.05$) mean excreta blood scores than birds fed either of the wheat diets, whether undosed or dosed.

Table 12. Mean probability estimates of blood in the excreta (probability ± SE of the probability) of undosed or dosed layers fed commercial feed or commercial or HP S1 wheat-based diets for 72 h at 25/26 weeks (n=8).

Caecal dosing	Diet		
	Commercial	Weston wheat	HP S2 wheat
Undosed	0.11 ± 0.083	0.13 ± 0.050	0.16 ± 0.075
Dosed	0.03 ± 0.130	0.09 ± 0.067	0.20 ± 0.070

Diarrhoea scores were initially very high (i.e. almost all the birds had wet excreta on the commercial diet) but were lowered ($p < 0.05$) when the birds were offered either wheat diet but increased over the 72 h of the trial to approach original values.

Short chain fatty acid concentration (mMol/L) (Table 13) in the caecal digesta was variously influenced by feed with propionic, *iso*-butyric, *iso*-valeric, n-valeric and hexanoic acids found in lower ($p < 0.05$) concentration in birds fed the commercial diet then the two wheat diets. Heptanoic acid was produced in lower ($p < 0.05$) concentration in birds dosed with caecal content at day-old. Acetic and total SCFA's were produced in greater ($p < 0.05$) concentration in the ileal digesta of birds on the commercial rather than the two wheat diets. Propionic acid in the ileal digesta was greater ($p < 0.05$) in commercial diet fed and dosed birds than in those on the other feed and dosing combinations while birds fed the HP S2 based feed and undosed at day-old produced no detectable propionic acid. The concentration of propionic acid was greater ($p < 0.05$) and *iso*-butyric acid lower ($p< 0.05$) in the jejunal digesta of birds fed the commercial diet than those given either of the wheat diets. Plasma acetic and total SCFA's were greater ($p < 0.05$) in

undosed birds and total SCFA's were greater ($p < 0.05$) in birds on the commercial diet. Feed type x caecal dosing interactions were produced in plasma propionic and *iso*-butyric acid concentrations with the commercial fed and dosed birds having more ($p < 0.05$) propionic acid than birds given the other combinations and birds fed the commercial diet, but dosed, having higher ($p < 0.05$) *iso*-butyric acid levels. In both cases, birds on both wheats and dosed had lower ($p < 0.05$) plasma concentrations.

The proportion (Table 14) of propionic, *iso*-butyric, *iso*- and n-valeric and hexanoic acids in caecal digesta was lower ($p < 0.05$) in birds fed the commercial rather than the wheat feeds. A greater ($p < 0.05$) proportion of hexanoic acid was produced in caecal digesta in birds that were dosed at day-old. In ileal digesta, more ($p < 0.05$) propionic acid was produced in birds fed the commercial diet and dosed at day-old than birds given the other feed and dosing combinations. More ($p < 0.05$) propionic acid was found in jejunal digesta in birds fed the commercial rather than the wheat diets. In plasma, acetic acid was in lesser ($p < 0.05$) and propionic acid in greater ($p < 0.05$) proportion in birds fed the commercial diet rather than the wheat diets. Birds dosed at day-old had greater ($p < 0.05$) proportions of acetic and propionic acids in plasma than those that were not dosed. *Iso*-butyric acid was in greater ($p < 0.05$) proportion in plasma in birds fed the commercial diet and dosed at day-old than those given the other diet and dosing combinations.

Histo-pathology of distal ileal tissue indicated that the three diets produced similar results i.e. no apparent differences in levels of inflammation were discernible. The differences between individual birds were, however, considerable.

A low concentration (4.0 ng ml-1) of myeloperoxidase was detected in the plasma of one bird. The bird was on the commercial wheat diet. No other plasma samples, from birds on any of the commercial, the commercial wheat blend or the HP S1 wheat diets contained sufficient MPO to exceed the detection limits.

Table 13. Influence of feeding layers a commercial feed or commercial or HP S1 wheat-based diets (n=8) for 72 h at 25/26 weeks on the short chain fatty acid (C2-C7) concentration (mMol) of digesta content in the mid-jejunum, distal ileum and caeca or plasma.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	Heptanoic	Total
Jejunum	Commercial	–	4.76	0.57	0	0.34	0	0	0	0.17	5.84
	Commercial	+	3.78	0.64	0	0.30	0.002	0.04	0	0.05	4.82
	Weston wheat	–	2.70	0.08	0	0.48	0	0.02	0	0.09	3.37
	Weston wheat	+	3.57	0.16	0	0.87	0.008	0	0.005	0.08	4.69
	HP S1 wheat	–	4.08	0.12	0	0.69	0	0.03	0	0.13	5.05
	HP S1 wheat	+	3.18	0.12	0	0.96	0	0.03	0	0.03	4.32
	SE		1.038	0.161		0.179	0.0039	0.025	0.0020	0.053	0.992
Ileum	Commercial	–	11.00	0.19 [b]	0.30	0.06	0.003	0	0	0.04	11.59
	Commercial	+	8.23	0.56 [a]	0.31	0.01	0.003	0.002	0	0.01	9.13
	Weston wheat	–	7.41	0.07 [bc]	0.30	0.34	0	0	0.010	0.01	8.16
	Weston wheat	+	5.87	0.08 [bc]	0	0.51	0	0	0	0.01	6.48
	HP S1 wheat	–	5.70	0 [c]	0.07	0.27	0	0	0.016	0	6.05
	HP S1 wheat	+	5.05	0.11 [bc]	0	0.61	0	0	0	0	5.78
	SE		1.178	0.059	0.158	0.224	0.0016	0.0010	0.0071	0.018	1.296
Caeca	Commercial	–	96.28	3.88	31.70	0.22	1.34	0.11	0.02	0.31	133.84
	Commercial	+	82.98	4.84	27.15	0.33	1.42	0.45	0.00	0.00	117.17
	Weston wheat	–	91.46	9.56	33.61	1.31	3.20	1.88	0.18	0.07	141.28
	Weston wheat	+	90.99	9.99	27.01	0.99	3.09	1.28	0.09	0.15	133.44
	HP S1 wheat	–	90.33	7.50	26.94	0.89	2.40	1.20	0.11	0.00	129.36
	HP S1 wheat	+	106.63	10.39	29.71	1.02	3.15	1.22	0.05	0.00	152.16
	SE		9.240	1.181	4.742	0.161	0.353	0.247	0.036	0.066	13.661
Plasma	Commercial	–	0.84	0.52 [a]	0	0.38 [b]	0	0	0	0	1.74
	Commercial	+	0.13	0.18 [b]	0.004	0.65 [a]	0	0	0	0	0.97
	Weston wheat	–	0.79	0.11 [bc]	0	0.41 [b]	0	0	0	0.05	1.36
	Weston wheat	+	0.30	0.02 [c]	0	0.20 [c]	0.007	0	0	0.02	0.55
	HP S1 wheat	–	0.70	0.12 [bc]	0	0.38 [bc]	0.007	0	0	0.01	1.21
	HP S1 wheat	+	0.26	0.00 [c]	0	0.21 [c]	0	0	0.007	0.04	0.52
	SE		0.110	0.044	0.0018	0.060	0.0042		0.0030	0.015	0.146

Table 14. Influence of feeding layers a commercial feed or commercial or HP S1 wheat-based diets (n=8) for 72 h at 25/26 weeks on the short chain fatty acid (C2-C7) proportion (%) of total SCFA in the digesta in the mid-jejunum, distal ileum and caeca or plasma.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Jejunum	Commercial	–	0.771	0.117	0	0.067	0	0	0	0.046
	Commercial	+	0.761	0.149	0	0.069	0.0004	0.007	0	0.014
	Weston wheat	–	0.750	0.022	0	0.188	0	0.008	0	0.033
	Weston wheat	+	0.703	0.030	0	0.244	0.0010	0	0.0016	0.021
	HP S1 wheat	–	0.727	0.016	0	0.203	0	0.006	0	0.048
	HP S1 wheat	+	0.730	0.036	0	0.220	0	0.007	0	0.007
	SE		0.0626	0.0175		0.0581	0.00047	0.0063	0.00063	0.0167
Ileum	Commercial	–	0.946	0.015 [b]	0.025	0.005	0.0007	0	0	0.0077
	Commercial	+	0.910	0.066 [a]	0.020	0.002	0.0001	0.0003	0	0.0021
	Weston wheat	–	0.886	0.011 [b]	0.041	0.059	0	0	0.0016	0.0016
	Weston wheat	+	0.877	0.014 [b]	0	0.106	0	0	0	0.0024
	HP S1 wheat	–	0.925	0.000 [b]	0.008	0.065	0	0	0.0018	0
	HP S1 wheat	+	0.878	0.022 [b]	0	0.100	0	0	0	0
	SE		0.0403	0.0098	0.0121	0.0418	0.00024	0.00011	0.00086	0.00322
Caeca	Commercial	–	0.726	0.031	0.227	0.002	0.010	0.001	0.0001	0.0024
	Commercial	+	0.707	0.048	0.222	0.004	0.013	0.001	0	0
	Weston wheat	–	0.653	0.068	0.231	0.010	0.023	0.014	0.0013	0.0004
	Weston wheat	+	0.689	0.072	0.200	0.007	0.022	0.010	0.0005	0.0001
	HP S1 wheat	–	0.704	0.061	0.196	0.008	0.019	0.011	0.0008	0
	HP S1 wheat	+	0.698	0.068	0.198	0.007	0.021	0.008	0.0003	0
	SE		0.0190	0.0068	0.0201	0.0013	0.0018	0.0024	0.00025	0.00056
Plasma	Commercial	–	0.456	0.299	0	0.245 [b]	0	0	0	0
	Commercial	+	0.126	0.176	0.005	0.693 [a]	0	0	0	0
	Weston wheat	–	0.565	0.081	0	0.303 [b]	0	0	0	0.052
	Weston wheat	+	0.499	0.027	0	0.442 [b]	0.012	0	0	0.021
	HP S1 wheat	–	0.570	0.099	0	0.319 [b]	0.006	0	0	0.006
	HP S1 wheat	+	0.509	0.000	0	0.407 [b]	0	0	0.015	0.069
	SE		0.0701	0.0297	0.0021	0.0763	0.0054		0.0060	0.0205

Fatty acid methyl esters in the ileal mucosa did not differ ($p > 0.05$) across the wheat diets or whether or not birds received a dose of caecal contents (Table 15).

Table 15. Fatty acid methyl esters (% total fatty acid) in the ileal mucosa of layers fed commercial or HP S1 wheat-based diets for 72 h at 25/26 weeks.

Fatty acid	Commercial wheat blend		HP S1		SE	
	Undosed [a]	Dosed [a]	Undosed [a]	Dosed [b]	n=8 [a]	n=7 [b]
C8:0	> 0.01	0	0	0.02	0.011	0.012
C10:0	0.03	0.04	0.03	0.03	0.001	0.010
C14:0	0.49	0.56	0.56	0.53	0.091	0.097
C14:1n-7	0.15	0.10	0.09	0.08	0.044	0.047
C16:0	23.09	23.05	22.87	22.09	0.574	0.613
C16:1n-7	2.62	3.75	3.50	3.11	0.365	0.390
C18:0	8.09	7.72	7.63	7.16	1.049	1.122
C18:1n-9	43.02	40.82	42.07	41.76	1.744	1.864
C18:1n-7	2.31	2.69	2.69	2.69	0.189	0.202
C18:2n-6	15.62	17.22	16.57	18.73	1.347	1.440
C18:3n-6	0.10	0.09	0.09	0.06	0.019	0.021
C18:3n-3	0.83	0.95	0.88	1.08	0.155	0.166
C20:0	0.18	0.11	0.18	0.15	0.057	0.061
C20:1n-9	0.33	0.34	0.42	0.43	0.047	0.050
C20:2n-6	0.05	0.08	0.07	0.05	0.015	0.017
C20:3n-6	0.14	0.06	0.10	0.08	0.041	0.044
C20:4n-6	2.08	1.14	1.40	1.03	0.853	0.912
C20:5n-3	0.17	0.19	0.05	0.14	0.085	0.091
C22:0	0.05	0.13	0.21	0.20	0.047	0.050
C22:1n-9	0.02	0.07	0.06	0.03	0.021	0.022
C22:2n-6	> 0.01	0.02	0	0	0.012	0.012
C22:3n-3	0.14	0.19	0.23	0.11	0.093	0.100
C22:5n-3	0.14	0.09	0.05	0.14	0.053	0.056
C22:6n-3	0.37	0.35	0.24	0.28	0.167	0.179
C24:0	> 0.01	0.02	> 0.01	0	0.008	0.009
C24:1n-9	0.01	0.01	0	0.02	0.009	0.009

Experiment 2

Body weights of the birds were similar ($p > 0.05$) at completion of the trial. Provision of the barley diet did not alter ($p > 0.05$) feed intake. A pre-trial a.m. excreta pH was not recorded for this experiment. The first pH measure (pm reading prior to the experiment) did not influence ($P > 0.05$) subsequent excreta pH. Mean excreta pH (Table 16) changed over the trial period with a higher ($p < 0.05$) pH at the pre-trial pm reading than for all subsequent readings and a consistently lower ($p < 0.05$) pH at the am than at the pm measurement as per the previous experiment.

Mean excreta pH was lower ($p < 0.05$) from birds given the barley diet (6.99 a and 6.51 b ± 0.071, commercial and barley fed birds respectively) and from birds undosed at day-old (6.59 b and 6.90 a ± 0.071 for undosed and dosed birds respectively). No time effects ($p > 0.05$) for feed or dosing were found for excreta pH changes (Table 16).

Log_{10} transformation of the excreta pH data provided a caecal dose x feed interaction with lower ($p < 0.05$) mean excreta pH from undosed birds on the barley diet than those on the commercial diet or dosed (Table 17).

Table 16. Mean excreta pH and excreta pH of layers fed commercial or barley-based diets (n=8) for 72 h at 37/38 weeks.

Diet	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Mean pH	Nil	7.58 a	6.26 c	7.13 b	6.23 c	6.96 b	6.23 c	7.15 b	6.44 c
SE					0.130				
Commercial	Nil	7.64	6.43	7.65	6.38	7.39	6.53	7.43	6.45
Barley	Nil	7.53	6.08	6.61	6.08	6.53	5.94	6.87	6.44
SE					0.202				
Undosed	Nil	7.55	5.97	7.15	6.17	6.86	6.18	6.85	6.04
Dosed	Nil	7.62	6.54	7.12	6.30	7.06	6.29	7.45	6.85
SE					0.202				

Table 17. Mean excreta pH of layers fed commercial or barley diets and either undosed or dosed (n=8) at 37/38 weeks.

Feed	Caecal dose	
	Undosed	Dosed
Weston commercial	6.93 [a]	7.04 [a]
Barley	6.26 [b]	6.76 [a]
SE	0.101	

Digesta and plasma L- or D-lactic acid in ileal or colonic digesta or plasma (Table 18) were unaltered ($p > 0.05$) by caecal dosing or feed change. Diet x dosing interactions of both caecal L- and D-lactate had undosed birds on the commercial diet having greater ($p < 0.05$) concentrations of each isomer than given the other diet / dosing treatments (Table 18).

Table 18. Digesta and plasma concentration of L- and D-lactic acid (mMol/L) of undosed or dosed layers fed a commercial or a barley-based diet (n=8) for 72 h at 37/38 weeks.

Source	Feed	Lactic acid (mMol)			
		L-lactic acid		D-lactic acid	
		Undosed	Dosed	Undosed	Dosed
Ileum	Commercial	21.18	10.20	17.09	6.97
	Barley	21.83	17.83	9.65	5.67
	SE	4.446		4.699	
Caeca	Commercial	5.63 [a]	1.57 [b]	5.90 [a]	1.79 [b]
	Barley	0.65 [b]	1.04 [b]	0.91 [b]	1.43 [b]
	SE	0.715		0.738	
Colon	Commercial	9.13	9.25	8.49	7.56
	Barley	16.28	11.28	11.75	5.15
	SE	4.283		4.477	
Plasma	Commercial	5.87	5.74	0.20	0.13
	Barley	6.77	6.25	0.06	0.05
	SE	0.507		0.058	

Total aerobe, total anaerobe and MRS (lactobacilli) counts (Table 19) were lower ($p < 0.05$) in the ileum than the caeca but were not altered ($p > 0.05$) by diet or dosing.

Table 19. Ileal and caecal digesta microbial counts (cfu ml^{-1}) of layers fed a commercial or a rice- based diet (n=8) for 72 h at 37/38 weeks.

Source	Feed	Microbial counts (cfu x 10^9) per ml of digesta					
		Total anaerobes		Total aerobes		Lactobacilli	
		Undosed	Dosed	Undosed	Dosed	Undosed	Dosed
Ileum	Commercial	3.61	2.24	2.83	0.58	3.46	1.93
	Barley	5.28	2.06	0.40	0.67	5.11	3.13
	SE	1.524×10^9		1.169×10^9		1.827×10^9	
Caeca	Commercial	9.48	10.73	5.56	7.36	7.49	4.36
	Barley	13.12	11.82	2.93	5.57	6.82	12.87
	SE	4.528×10^9		3.081×10^9		3.242×10^9	

Blood was not detected in excreta during random checks prior to the test diets being fed to the birds. A modest increase ($p < 0.05$) in blood (Table 20) was found over the 72 hrs of the experiment but with no influence of diet or dosing ($p > 0.05$). However, comparison of the feeds over time indicated that the dietary effect approached significance ($z = -1.906$, $p = 0.057$) with the barley diet causing a consistently higher excreta blood score from early in the trial period.

Table 20. Probability estimates of blood in the excreta (probability ± SE of the probability) of layers fed a commercial or a barley-based diet for 72 h at 37/38 weeks and birds undosed or dosed with caecal contents at day-old (n=8).

Feed		Time (h)							
	-24	-12	0	12	24	36	48	60	72
Commercial	Nil	0.00 ± 1.521	0.00 ± 1.292	0.00 ± 1.065	0.00 ± 0.838	0.00 ± 0.615	0.02 ± 0.398	0.08 ± 0.210	0.28 ± 0.186
Barley	Nil	0.01 ± 0.334	0.02 ± 0.277	0.03± 0.223	0.05 ± 0.173	0.08 ± 0.132	0.12 ± 0.112	0.18 ± 0.121	0.25 ± 0.155
Undosed	Nil	0.00 ± 0.441	0.01 ± 0.369	0.02 ± 0.298	0.03 ± 0.230	0.06 ± 0.169	0.11 ± 0.124	0.20 ± 0.117	0.33 ± 0.151
Dosed	Nil	0.00 ± 0.525	0.01 ± 0.439	0.01 ± 0.355	0.02 ± 0.275	0.03 ± 0.205	0.06 ± 0.158	0.09 ± 0.156	0.15 ± 0.200

Diarrhoea scores were again quite high (i.e. many birds having wet excreta on the commercial diet) and mean probability estimates were higher ($z = 2.198$, $p = 0.028$) with dosed versus undosed birds. Diet did not alter ($p > 0.05$) diarrhoea scores.

Short chain fatty acid concentration (mMol/L) (Table 21) was not altered ($p > 0.05$) in the caecal digesta. *Iso*-butyric acid was greater ($p < 0.05$) in ileal digesta of birds dosed at day-old (0.089 and 0.293 ± 0.0359 for undosed and dosed birds respectively). In the colon, $\log_{10}$ transformation produced a greater ($p < 0.05$) acetic acid concentration in dosed birds (7.25 and 25.92 ± 5.050 for undosed and dosed birds respectively) and a greater total VFA concentration in dosed birds approached significance ($p = 0.054$), due largely to the acetic acid content. Plasma acetic acid concentration was greater ($p < 0.05$) in the undosed birds on the commercial diet than those dosed or given the barley diet and the undosed birds on the barley diet had a higher ($p < 0.05$) concentration than dosed birds on either diet (1.14 a, 0.17 c, 0.49 b and 0.09 c ± 0.094 for commercial undosed and dosed and barley undosed and dosed respectively). Total plasma VFA's were higher ($p < 0.05$) in birds fed the commercial than those fed the barley diet (1.33 a and 0.66 b ± 0.201) undosed birds than those that were dosed (1.44 a and 0.55 b ± 0.201).

The proportions of each short chain fatty acid (Table 22) were not influenced ($p > 0.05$) by diet or caecal dosing in any gut section although *iso*-butyric acid in colonic digesta of undosed birds approached a significantly greater ($p = 0.058$) proportion than in those birds that were dosed. Plasma VFA proportions were not calculated (see Discussion below).

Histo-pathology of distal ileal tissue again showed similar results from both diets i.e. no apparent differences in levels of inflammation were discernible. Myeloperoxidase concentrations in the plasma of birds on either the commercial or barley diets did not exceed the detection limits.

Table 21. Influence of feeding undosed or dosed layers a commercial or a barley-based diet (n=8) for 72 h at 37/38 weeks on the short chain fatty acid (C2-C7) concentration (mMol) of digesta content in the distal ileum, caeca and colon or plasma.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)								
			acetic	Propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	Hexanoic	heptanoic	Total
Ileum	Commercial	–	5.47	0.08	0	0.05	0	0	0.01	0.06	5.67
	Commercial	+	4.68	0	0.20	0.29	0.01	0	0	0.02	5.21
	Barley	–	3.85	0.10	0.17	0.13	0	0	0	0	4.24
	Barley	+	6.30	0.27	0.13	0.30	0	0	0	0	7.00
	SE		0.815	0.077	0.101	0.051	0.007		0.007	0.031	0.861
Caeca	Commercial	–	63.83	4.44	21.18	0.37	1.70	0.31	0.05	0.09	91.96
	Commercial	+	83.58	7.29	24.75	0.57	1.27	0.66	0.01	0	118.12
	Barley	–	76.42	3.19	12.61	0.31	1.21	0.31	0	0	94.04
	Barley	+	81.64	6.78	19.10	0.53	1.13	0.65	0	0	109.83
	SE		8.770	1.689	5.330	0.151	0.742	0.241	0.025	0.026	12.363
Colon	Commercial	–	7.45	0.22	0.72	0.25	0.04	0	0	0	8.67
	Commercial	+	32.41	1.13	6.32	0.14	0.23	0.03	0.01	0	40.28
	Barley	–	7.05	0.38	0.52	0.14	0.03	0	0	0	8.12
	Barley	+	19.43	0.62	3.74	0.23	0.01	0	0	0	24.04
	SE		7.142	0.320	2.184	0.036	0.094	0.014	0.007		9.529
Plasma	Commercial	–	1.14 [a]	0.19	0.12	0.06	0.07	0.07	0.10	0.31	2.05
	Commercial	+	0.17 [c]	0	0	0.15	0	0	0	0.29	0.60
	Barley	–	0.49 [b]	0	0	0.05	0	0	0	0.29	0.83
	Barley	+	0.09 [c]	0	0	0.11	0	0	0.01	0.28	0.49
	SE		0.094	0.046	0.032	0.038	0.035	0.033	0.035	0.022	0.284

Table 22. Influence of feeding undosed or dosed layers a commercial or a barley-based diet (n=8) for 72 h at 37/38 weeks on the short chain fatty acid (C2-C7) proportion (%) of total SCFA in the digesta in the mid-jejunum, distal ileum and caeca.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)							
			Acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Ileum	Commercial	–	0.965	0.014	0	0.011	0	0	0.001	0.010
	Commercial	+	0.898	0	0.039	0.055	0.003	0	0	0.005
	Barley	–	0.928	0.015	0.025	0.032	0	0	0	0
	Barley	+	0.898	0.040	0.019	0.042	0	0	0	0
	SE		0.0280	0.0120	0.0156	0.0128	0.0015		0.0006	0.0053
Caeca	Commercial	–	0.694	0.049	0.230	0.004	0.018	0.004	0.001	0.001
	Commercial	+	0.704	0.064	0.209	0.005	0.012	0.006	0	0
	Barley	–	0.845	0.030	0.106	0.004	0.012	0.004	0	0
	Barley	+	0.749	0.066	0.163	0.005	0.010	0.007	0	0
	SE		0.0488	0.0161	0.0425	0.0017	0.0069	0.0027	0.0003	0.0004
Colon	Commercial	–	0.881	0.023	0.061	0.033	0.002	0	0	0
	Commercial	+	0.848	0.022	0.117	0.010	0.003	0.001	0.0003	0
	Barley	–	0.885	0.048	0.046	0.019	0.002	0	0	0
	Barley	+	0.851	0.033	0.103	0.013	0.001	0	0	0
	SE		0.0327	0.0091	0.0348	0.0057	0.0021	0.0003	0.00013	

Fatty acid methyl esters in the ileal mucosa did not differ ($p > 0.05$) across the diets other than for a higher ($p < 0.05$) proportion of C18:2n-6 when birds continued on the commercial feed and had remained undosed at day old. Undosed birds had a higher ($p < 0.05$) proportion of C20:3n-6 than dosed birds (Table 23).

Table 23. Fatty acid methyl esters (% total fatty acid) in the ileal mucosa of layers fed commercial or barley-based diets for 72 h at 37/38 weeks.

Fatty acid	Commercial diet		Barley		SE	
	Undosed [a]	Dosed [b]	Undosed [b]	Dosed [b]	n=3 [a]	n=4 [b]
C8:0	0	0	0	0	0	0
C10:0	0	0.01	0	0.01	0.011	0.010
C14:0	0.33	0.51	0.52	0.46	0.069	0.061
C14:1n-7	0.33	0.20	0.27	0.41	0.160	0.139
C16:0	17.84	20.29	19.77	18.98	1.180	1.022
C16:1n-7	1.17	2.40	1.72	1.74	0.558	0.483
C18:0	14.57	10.06	13.70	13.45	2.031	1.759
C18:1n-9	25.72	35.53	33.00	32.18	3.325	2.880
C18:1n-7	2.54	2.61	2.42	2.47	0.168	0.146
C18:2n-6	26.27 [a]	19.46 [b]	18.37 [b]	19.28 [b]	1.490	1.290
C18:3n-6	0.12	0.12	0.10	0.17	0.086	0.074
C18:3n-3	0.98	0.93	0.71	0.68	0.132	0.115
C20:0	0.83	0.45	0.62	0.52	0.163	0.142
C20:1n-9	1.04	0.42	0.39	0.35	0.296	0.256
C20:2n-6	0	0.09	0.02	0.09	0.031	0.027
C20:3n-6	0.31 [a]	0.11 [b]	0.33 [a]	0.26 [b]	0.050	0.043
C20:4n-6	4.27	2.62	4.10	4.29	0.985	0.853
C20:5n-3	0.47	0.28	0.29	0.42	0.108	0.093
C22:0	0.27	0.32	0.40	0.34	0.126	0.109
C22:1n-9	0	0.02	0.05	0	0.031	0.027
C22:2n-6	0	0.13	0.04	0.14	0.087	0.076
C22:3n-3	0.65	0.26	0.24	0.21	0.186	0.161
C22:5n-3	0.05	0.20	0.24	0.08	0.095	0.082
C22:6n-3	1.39	0.59	1.25	1.32	0.362	0.314
C24:0	0	0.10	0.16	0.03	0.085	0.074
C24:1n-9	0.84	2.30	1.31	2.15	1.161	1.006

Experiment 3

There was no alteration ($p > 0.05$) in feed intake with the change to a rice diet. The first pH measure (a.m. reading at - 24 h) influenced ($p < 0.05$) subsequent excreta pH. Mean excreta pH (Table 24) was higher ($p < 0.05$) at the p.m. than a.m. reading. Excreta pH (Table 24) was reduced ($p < 0.05$) by the rice diet over time from 12 h after the feed was presented to the birds. Dosing at day-old did not influence ($p > 0.05$) excreta pH.

Digesta and plasma L- or D-lactic acid concentrations in caecal or colonic digesta or plasma (Table 25) were unaltered ($p > 0.05$) by caecal dosing or feed change. Ileal D-lactate was not influenced ($p > 0.05$) by diet or dosing but ileal L-lactate increased ($p < 0.05$) with consumption of the rice diet (20.4 b ± 4.022 and 42.9 a ± 4.163 for commercial and rice diets respectively).

Total aerobe, total anaerobe and MRS (lactobacilli) counts (Table 26) were lower ($p < 0.05$) in the ileum than the caeca but were not altered ($p > 0.05$) by diet or dosing. However, log transformed data of both caecal aerobe and anaerobe counts approached significance ($p = 0.068$ and 0.089 respectively). In each case a feed x dose interaction indicated that dosed birds fed the commercial diet had lower counts than undosed birds on the commercial diet and dosed birds on the rice diet but similar counts to the undosed birds on the rice diet.

Table 24. Mean excreta pH and excreta pH of layers fed a commercial or a rice-based diet (n=8) for 72 h at 42/43 weeks.

Diet	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Mean pH	6.27	7.79 a	6.49 c	7.12 b	6.23 c	6.98 b	6.36 c	7.11 b	6.28 c
SE					0.117				
Commercial	6.27	7.84 a	6.58 c	7.58 ab	6.55 cd	7.36 b	6.65 c	7.56 ab	6.44 cd
Barley	6.27	7.75 a	6.40 cd	6.66 c	5.91 f	6.60 c	6.07 ef	6.67 c	6.11 de
SE					0.169				
Undosed	6.36	7.78	6.42	7.19	6.17	7.04	6.32	7.07	6.25
Dosed	6.18	7.81	6.56	7.06	6.30	6.92	6.41	7.16	6.30
SE					0.152				

Table 25. Digesta and plasma concentration of L- and D-lactic acid (mMol/L) of undosed or dosed layers fed a commercial or a rice-based diet (n=8) for 72 h at 42/43 weeks.

Source	Feed	Lactic acid (mMol)			
		L-lactic acid		D-lactic acid	
		Undosed	Dosed	Undosed	Dosed
Ileum	Commercial	21.79 [b]	19.08 [b]	13.83	9.69
	Rice	36.32 [a]	49.41 [a]	14.03	23.73
	SE	6.080		4.961	
Caeca	Commercial	2.58	2.20	3.16	2.40
	Rice	2.86	3.54	3.81	4.71
	SE	0.518		0.703	
Colon	Commercial	23.37	20.49 ± 5.806	17.95	11.53 ± 5.062
	Rice	31.79	26.50 ± 8.212	16.60	24.15 ± 7.159
	SE	6.705		5.845	
Plasma	Commercial	5.12	5.14	0.13 ± 0.388	0.14 ± 0.448
	Rice	5.40	5.05	0.04 ± 0.448	1.16 ± 0.490
	SE	0.481	0.589		

Table 26. Ileal and caecal digesta microbial counts (cfu ml^{-1}) of layers fed a commercial or a rice-based diet (n=8) for 72 h at 42/43 weeks.

Source	Feed	Microbial counts (cfu x 10^9) per ml of digesta					
		Total anaerobes		Total aerobes		Lactobacilli	
		Undosed	Dosed	Undosed	Dosed	Undosed	Dosed
Ileum	Commercial	6.40	3.12	2.64	0.70	8.14	3.40
	Rice	6.14	13.33	3.38	6.12	6.35	10.14
	SE	3.579×10^{10}		2.154×10^9		3.254×10^9	
Caeca	Commercial	63.03	4.85	15.88	1.74	20.93	10.12
	Rice	7.98	31.51	3.28	16.54	6.83	22.53
	SE	2.257×10^{10}		5.635×10^9		5.245×10^9	

Blood was not detected in excreta during random checks prior to the test diets being fed to the birds. Distinct dose and feed effects were found for blood scores (Table 27) with undosed birds maintaining a lower ($p < 0.05$) blood score than dosed birds and rice fed birds a higher ($p < 0.05$) score than birds fed the commercial diet. Overall mean blood scores increased ($p < 0.05$) over the experiment but a time x feed interaction approached significance ($p = 0.104$).

Table 27. Probability estimates of blood in the excreta (probability ± SE of the probability) of layers fed a commercial or a rice-based diet for 72 h at 42/43 weeks and birds undosed or dosed with caecal contents at day-old (n=8).

Feed	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Commercial	0.01 ± 0.346	0.01 ± 0.290	0.01 ± 0.239	0.01 ± 0.199	0.01 ± 0.176	0.01 ± 0.178	0.02 ± 0.204	0.02 ± 0.246	0.02 ± 0.298
Rice	0.02 ± 0.171	0.03 ± 0.144	0.05 ± 0.119	0.09 ± 0.095	0.14 ± 0.074	0.21 ± 0.060	0.30 ± 0.057	0.42 ± 0.066	0.55 ± 0.082
Undosed	0.01 ± 0.285	0.01 ± 0.243	0.02 ± 0.202	0.03 ± 0.163	0.04 ± 0.128	0.06 ± 0.101	0.09 ± 0.088	0.13 ± 0.097	0.19 ± 0.122
Dosed	0.02 ± 0.174	0.04 ± 0.147	0.05 ± 0.122	0.08± 0.098	0.11 ± 0.077	0.16 ± 0.064	0.22 ± 0.062	0.30 ± 0.071	0.39 ± 0.088

Basal diarrhoea scores (Table 28) remained high (i.e. many birds producing wet excreta on the commercial diet) and mean probability estimates were higher ($z = 3.628$, $p = 0.0003$) from birds on the rice rather than the commercial diet. Dosing did not alter ($p > 0.05$) diarrhoea scores (Table 28).

Table 28. Probability estimates of diarrhoea (probability ± SE of the probability) with layers fed a commercial or a rice-based diet for 72 h at 42/43 weeks and birds undosed or dosed with caecal contents at day-old (n=8).

Feed	Time (h)								
	-24	-12	0	12	24	36	48	60	72
Commercial	0.34 ± 0.079	0.33 ± 0.066	0.33 ± 0.055	0.32 ± 0.046	0.32 ± 0.044	0.32 ± 0.047	0.31 ± 0.056	0.31 ± 0.067	0.30 ± 0.081
Rice	0.44 ± 0.080	0.54 ± 0.063	0.63 ± 0.050	0.72 ± 0.046	0.79 ± 0.050	0.85 ± 0.061	0.90 ± 0.074	0.93 ± 0.089	0.95 ± 0.0105
Undosed	0.46 ± 0.073	0.48 ± 0.061	0.51 ± 0.050	0.54 ± 0.042	0.56 ± 0.039	0.59 ± 0.042	0.62 ± 0.050	0.64 ± 0.060	0.66 ± 0.072
Dosed	0.37 ± 0.076	0.40 ± 0.063	0.44 ± 0.051	0.47 ± 0.043	0.51 ± 0.040	0.54 ± 0.042	0.58 ± 0.050	0.61 ± 0.060	0.64 ± 0.071

Short chain fatty acid concentration (mMol/L) (Table 29) in the caecal digesta was influenced by feed with greater ($p < 0.05$) acetic and total acid concentration from commercial fed birds, greater ($p < 0.05$) *iso*-valeric acid concentration from rice-fed birds and a greater ($p < 0.05$) n-butyric acid concentration in birds fed the commercial diet and dosed at day-old. No differences ($p > 0.05$) in VFA concentrations were found in ileal or colonic digesta. Serum acetic and propionic acids and total fatty acid concentrations were greater ($p < 0.05$) in the undosed birds than those dosed at day-old. *Iso*-butyric acid displayed a dose x feed interaction with commercial-fed, undosed birds having detectable ($p < 0.05$) concentrations while birds on the other diet/dosing combinations had none.

The proportions of each short chain fatty acid (Table 30) were not influenced ($p > 0.05$) by diet or caecal dosing in the ileum and colon. Caecal digesta *iso*-butyric and *iso*-valeric acid proportions were greater ($p < 0.05$) in rice than commercial-fed birds. Serum acetic and propionic acid proportions were greater ($p < 0.05$) in undosed birds, heptanoic acid was greater ($p < 0.05$) in dosed birds while *iso*-butyric acid was only found in the commercial-fed, undosed birds.

Histo-pathology of distal ileal tissue indicated a difference in inflammation with the birds on the rice diet exhibiting a greater response.

Low concentrations of myeloperoxidase were detected in the plasma of four birds. Three birds had remained on the commercial feed and only one bird had been changed to the rice feed.

Table 29. Influence of feeding undosed or dosed layers a commercial or a rice-based diet (n=8) for 72 h at 42 / 43 weeks on the short chain fatty acid (C2-C7) concentration (mMol) of digesta content in the distal ileum, caeca and colon or plasma.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Commercial	–	5.12	0.08	0.18	0.06	0	0.002	0	0	5.43
	Commercial	+	4.82	0.07	0.01	0.11	0	0	0	0.04	5.04
	Rice	–	5.66	0.05	0.05	0.07	0	0	0	0.04	5.87
	Rice	+	5.46	0.07	0.10	0.10	0	0	0.005	0.04	5.77
	SE		0.986	0.029	0.077	0.020		0.0009	0.0026	0.025	1.029
Caeca	Commercial	–	83.60	3.56	15.65 [b]	0.37	0.97	0.48	0.06	0.08	104.76
	Commercial	+	98.44	4.43	30.36 [a]	0.34	0.95	0.26	0	0	134.77
	Rice	–	62.04	4.32	15.44 [b]	0.73	1.64	0.96	0.04	0.12	85.29
	Rice	+	56.82	4.19	9.11 [b]	0.84	1.56	1.27	0.03	0.04	73.85
	SE		10.578	0.642	4.291	0.129	0.371	0.184	0.020	0.039	12.883
Colon	Commercial	–	7.25	0.19	0.48	0.08	0	0	0	0.05	8.04
	Commercial	+	17.19	0.55	5.45	0.03	0.12	0.01	0	0.04	23.40
	Rice	–	8.98	0.22	0.42	0.07	0.05	0.01	0	0	9.76
	Rice	+	27.45	1.46	6.31	0.09	0.44	0.09	0	0	35.85
	SE		9.847	0.496	4.780	0.019	0.240	0.027		0.030	15.199
Serum	Commercial	–	0.75	0.08	0.04	0.03 [a]	0	0.015	0	0.14	1.06
	Commercial	+	0.18	0.01	0	0 [b]	0	0.001	0.005	0.21	0.40
	Rice	–	0.58	0.08	0.02	0 [b]	0	0	0	0.13	0.80
	Rice	+	0.16	0	0	0 [b]	0	0	0.008	0.21	0.38
	SE		0.148	0.021	0.019	0.005		0.0075	0.0038	0.025	0.162

Table 30. Influence of feeding undosed or dosed layers a commercial or a rice-based diet (n=8) for 72 h at 42 / 43 weeks on the short chain fatty acid (C2-C7) proportion (%) of total SCFA in the digesta in the mid-jejunum, distal ileum and caeca.

Organ	Feed	Dose	Short chain fatty acid (C2-C7)							
			Acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	Hexanoic	Heptanoic
Ileum	Commercial	–	0.942	0.011	0.032	0.014	0	0.001	0	0
	Commercial	+	0.953	0.014	0.014	0.019	0	0	0	0.015
	Rice	–	0.958	0.008	0.025	0.015	0	0	0	0.007
	Rice	+	0.944	0.012	0.011	0.028	0	0	0.001	0.005
	SE		0.0114	0.0043	0.0094	0.0048		0.0004	0.0003	0.0063
Caeca	Commercial	–	0.792	0.039	0.145	0.005	0.011	0.007	0.0005	0.0005
	Commercial	+	0.740	0.041	0.207	0.004	0.007	0.015	0	0
	Rice	–	0.742	0.059	0.150	0.011	0.022	0.004	0.0005	0.0013
	Rice	+	0.734	0.054	0.154	0.014	0.022	0.021	0.0004	0.0009
	SE		0.0347	0.0094	0.0320	0.0023	0.0054	0.0032	0.00023	0.00043
Colon	Commercial	–	0.897	0.031	0.045	0.016	0	0	0	0.011
	Commercial	+	0.904	0.031	0.054	0.004	0.001	0	0	0.008
	Rice	–	0.947	0.019	0.025	0.006	0.003	0.001	0	0
	Rice	+	0.849	0.039	0.099	0.006	0.005	0.002	0	0
	SE		0.0418	0.0059	0.0403	0.0037	0.0019	0.0006		0.0068
Serum	Commercial	–	0.526	0.072	0.029	0.020	0	0.018	0	0.335
	Commercial	+	0.304	0.024	0	0	0	0.002	0.009	0.662
	Rice	–	0.467	0.227	0.023	0	0	0	0	0.284
	Rice	+	0.297	0	0	0	0	0	0.022	0.682
	SE		0.0695	0.0621	0.0152	0.0038		0.0098	0.0091	0.1018

Fatty acid methyl esters in the ileal mucosa did not differ ($p > 0.05$) across the diets or whether or not birds received a dose of caecal contents (Table 31).

Table 31. Fatty acid methyl esters (% total fatty acid) in the ileal mucosa of layers fed commercial or rice-based diets for 72 h at 42/43 weeks.

Fatty acid	Commercial diet		Rice		SE
	Undosed	Dosed	Undosed	Dosed	N=4
C8:0	0	0	0	0	0
C10:0	0	0	0	0	0
C14:0	0.29	0.44	0.49	0.34	0.097
C14:1n-7	0.15	0.23	0.40	0.30	0.164
C16:0	16.69	19.17	16.97	16.10	1.330
C16:1n-7	0.92	1.91	1.44	1.17	0.446
C18:0	18.38	12.57	13.33	15.00	2.485
C18:1n-9	28.11	32.02	26.64	26.86	3.659
C18:1n-7	2.30	2.56	2.37	2.08	0.204
C18:2n-6	24.56	19.51	18.62	18.46	2.557
C18:3n-6	0.13	0.15	0.09	0.27	0.098
C18:3n-3	0.58	0.86	0.85	0.58	0.189
C20:0	0.47	0.51	0.76	0.54	0.164
C20:1n-9	0.15	0.32	0.31	0.25	0.128
C20:2n-6	0.03	0.07	0	0.03	0.031
C20:3n-6	0.15	0.26	0.06	0.25	0.082
C20:4n-6	4.71	3.86	4.07	4.75	0.984
C20:5n-3	0.44	0.30	0.91	0.71	0.174
C22:0	0.14	0.39	0.27	0.35	0.099
C22:1n-9	0	0.17	0.16	0	0.105
C22:2n-6	0.20	0.09	0	0.28	0.114
C22:3n-3	0.12	0.37	0.60	0.26	0.139
C22:5n-3	0.10	0.13	0.10	0.25	0.086
C22:6n-3	1.12	1.12	1.46	1.53	0.377
C24:0	0.17	0.14	0.13	0.57	0.179
C24:1n-9	0.08	2.89	9.99	9.10	4.924

4.4 Discussion

As noted in earlier (Taylor, 2002) work, in laying stock excreta pH displayed a clear diurnal pattern of low morning and high evening pH. Each of the three experiments with wheat, barley and rice diets produced this effect which was similar in the commercial diet. With a long dark period prior to the morning excreta pH reading, diurnal differences in the digestive process are highlighted. Ammonia is the predominant alkalinising component in the hindgut (Newmark and Lupton, 1990) and the reduced pH of the rice diet could suggest differences in ammonia concentration that was reduced particularly at the evening reading. However, Williams *et al*. (1997) indicated that there was no difference in ammonia levels present at the end of dietary fermentation that were due to differences in dietary N contents. Therefore, the contribution of other digesta/excreta components to pH must be considered. As the greater pH difference between the commercial and test diets was found at the evening reading, at a time when digestive processes including fermentation would be active in the later part of the days feeding, the fermentation metabolites should be considered. In each experiment there was a difference in the pH effect across cereals. The two test wheat diets had little major effect on excreta pH, the barley diet reduced the mean pH over the 72 h test period but the rice diet produced a substantial pH reduction. Of the organic acids, there was little change in SCFA concentrations or proportions across the experiments. Some reduction in caecal acetic acid concentration, but not proportion, was effected by feeding a rice diet. Mead (1989) noted that the predominant end products of uric acid breakdown in the chicken hindgut were acetate, CO_2 and ammonia. As ammonia has been discounted as affecting the pH difference, and acetate levels were reduced on the rice diet, the contribution of lactic acid to total acidity must be considered. Carre *et al*. (1995) showed that lactic acid contributed the major proportion of organic acid losses in the excreta of layer strain cockerels.

Lactic acid concentrations trended higher when the wheat and barley diets were fed to the birds. This was evident in the ileal digesta and was also found in the rice trial with substantial increases in L-lactic acid concentration.

Blood loss was noted in the excreta when each cereal-based feed was substituted for the commercial diet. The noted problem wheat, HP S1 caused a sudden and substantial increase in blood loss compared with the commercial wheat blend which took more than 48 h to cause a similar response. The response to the barley diet, with a mean increase in blood loss ($p=0.057$), was masked, given the limited replication in the experiment, by three birds on the commercial feed returning a single positive result each over the 72 h of the experiment. It was when the rice diet was fed that blood loss was rapid and consistent across the birds. Diarrhoea was a feature associated with the blood loss when the rice diet was fed; a symptom that was not associated with the response to the wheat and barley by most birds.

The pH reduction, ileal L-lactic acid and blood production and diarrhoea associated with the rice diet support the argument that a lactic acidosis was produced with substitution of a new cereal base to the diet fed to layers. As L-lactic acid concentration was increased greatly, its production from the ileal mucosa must be considered. In human ulcerative colitis, a doubled production of lactic acid from the colonic epithelium could enter the gut lumen (Roediger, 1989). The ileal mucosa of the rat is more sensitive than that of the colon to high concentrations of H^+ and lactate (Saunders and Sillery, 1982). Fresh blood entering the gut lumen would increase luminal oxygen content and favour the activity of facultative Lactobacilli (Vernia *et al*., 1988a). These factors are all supported by the results of commercial histopathology (Rod Reece, NSW Agriculture) which indicated that birds on the rice diet had indications of greater ileal inflammation including larger and more numerous lymphoid foci.

Microbial counts were compromised by the low replication although both test wheats decreased ileal aerobes and increased anaerobes relative to the commercial feed. Neither barley nor rice diets altered microbial counts.

The microbial counts highlighted the confounding factor of caecal dosing applied to the birds at day old. Resources available to the project precluded replication of rearing housing based on caecal dosing. The birds were maintained in replicated units in the brooders following dosing but single floor pens were used to grow the groups as dosed or undosed, until the birds were used in the feed trials. Nevertheless, analysis had to include caecal dosing as a main effect. Some interactions were observed with microbial counts in the wheat experiment, excreta pH measures on the barley diet and, importantly, dosed birds produced higher blood scores during the rice trial and more diarrhoea during the barley trial. The main observation of the dosing was that throughout the rearing period and into lay, the undosed birds suffered from bouts of picking, with mortality, whilst the dosed birds did not. Certainly, this observation would require replicated analysis to be considered seriously.

The markers of lactic or fermentative acidosis, pH reduction and increases in lactic acid concentration were found consistently through these experiments when the birds were changed from their accustomed diet to one based on different cereals. When pH and lactic acid concentration changed markedly, with feeding of a rice diet, adverse hindgut responses were noted, particularly in the ileum. Perhaps the condition could be better described as an ileitis resulting from diet change.

5. Long-term “colitis-like” response to diet in commercial layers.

5.1 Introduction

The experiments reported above indicate that dietary cereal change causes an immediate response from the gut with blood loss in the excreta being found, diarrhoea being generally associated with the blood loss and some indication that hindgut mucosae, particularly of the distal ileum, display inflammation. The response to different diets in the long term required consideration.

A large data set produced during earlier work (Taylor, 1998) was available for analysis of the effects of dietary differences, across two strains of laying hens and under the influence of different feed methods. A method of ascribing scores to descriptive data and for subsequent analysis was investigated and tested and was deemed appropriate application to the data set.

5.2 Materials and methods

Birds, feeds and feeding methods were described by Taylor (1998). SIRO CB and Hy-Line Brown layers were fed a wheat-based diet as either a whole grain or as a ground and crumbled feed. The birds were offered diets containing either ground limestone as the calcium source or the limestone was offered separately as a whole grit either daily or every second day.

Six sets of excreta collections, each set over two consecutive days to allow for days on and off the limestone grit for birds on the intermittent grit treatment, were made from laying hens between 24 and 51 weeks of age. The excreta for each day were collected over a full 24 h into foil-lined trays attached to the bottom of single-bird cages. The excreta were described then washed through a series of sieves to collect excreta fractions for further measurement. For this experiment, the presence or absence of fresh blood was scored. These binary data were analysed as described above (2.4 Statistical analyses).

5.3 Results

The method of presentation of the wheat, as either a whole grain or a ground and crumbled feed, altered blood loss in the excreta with whole grain producing a higher ($p < 0.05$) mean score than the ground and crumbled grain (Table 32). However, the strain of layer and grain processing interacted (Table 33) with the SIRO CB layers fed the whole wheat producing a higher ($p < 0.05$) mean blood score than their counterparts fed the ground and crumbled wheat or the Hy-Line Brown birds fed either form of the wheat. Providing calcium, as either a ground limestone or a large grit, or with intermittent provision of the grit had no effect ($p > 0.05$) on blood loss in the excreta of the birds.

Table 32. Probability estimates (± SE of the probability) of blood in the excreta of 24 – 51 week old layers fed a wheat-based diet as either whole grain or a ground and crumbled feed.

	Age (weeks)					
Wheat diet	24	26	30	34	39	51
Ground and crumbled[1]	0.31 0.064	0.30 0.048	0.30 0.038	0.30 0.038	0.30 0.048	0.29 0.065
Whole grain	0.44 0.058	0.42 0.044	0.40 0.035	0.38 0.035	0.37 0.045	0.35 0.061

[1] n = 72 birds on either diet

Table 33. Probability estimates (± SE of the probability) of blood in the excreta of Hy-Line Brown or SIRO CB layers fed a wheat-based diet as either whole grain or a ground and crumbled feed.

Wheat diet	Layer strain	
	Hy-Line Brown	SIRO CB
Ground and crumbled[1]	0.31 [b]	0.29 [b]
	0.051	0.052
Whole grain	0.31 [b]	0.48 [a]
	0.051	0.045

[1] n = 36 birds of either strain on either diet.

5.4 Discussion

It was important to establish that dietary differences could alter the pattern of blood loss over the long term. Despite the same grain being fed to the birds from the change to a layer diet at 18 weeks of age, successive batches of wheat were used. It is recognised that grain changes over the storage period and this may have had an effect on the hindgut processes. The greater blood loss in the excreta of the SIRO CB birds fed the whole grain may highlight the effect of cereal grains on the hindgut when processing is different. The SIRO CB birds ate much greater quantities of grain to meet their energy requirements. These birds had lower performance than the Hy-Line Brown strain and overall the SIRO CB had a much poorer energetic efficiency but only in the laying phase (Taylor, 1998). The greater grain intake was associated with a greater 'colitis-like' response only when a different form of grain processing was applied and to mature layers. Recent work (Taylor and Jones, 2004a; Taylor and Jones, 2004b) has shown that a modest change in grain processing, with a mere 200 g kg^{-1} of the cereal component included into the feed mix as a whole grain prior to pelleting, had a marked effect upon gut development in broilers. The effects, both physical and chemical, resulted in proventricular dilatation being ameliorated on wheat-based diets and completed negated with barley inclusion in a sorghum-based feed. This was associated with an overall reduction in ascites mortality across the range of different cereals. A simple physical effect was not considered fully explanatory for the whole response as changes in digesta flow through the fore- and hindgut were noted and digesta pH was altered. These effects accrued from whole grain inclusion and with lesser influence deriving from the application of an exogenous feed enzyme.

6. Digesta characteristics and "colitis-like" responses after dietary cereal changes and inclusion of antibiotics to broilers.

6.1 Introduction

In earlier work (Taylor, 2002) broiler trials were added to monitor changes in hindgut function in another bird type. The results provided some indication that similar responses to diet change occurred in broiler and layer birds. With its relatively short production cycle and the apparently greater enteric disease problems associated with its production, the broiler appeared to be the ideal type in which to monitor effects of diet change with or without antimicrobial use.

The following experiments were conducted with a range of cereals and with application of virginiamycin and avilamycin.

6.2 Materials and Methods

Birds and experimental design

One-d-old male broiler chickens (Ross x Ross; Ross 308) were obtained from a commercial hatchery and housed in small electrically-heated brooders in an environmentally-controlled, continuously-lit room until 5 d of age. They were offered commercial broiler starter crumbles (12.5 MJ ME/kg and 220 g crude protein (CP)/kg) and water *ad libitum*. At 5 d of age, the birds were individually weighed and allocated in groups of six to the brooders.

The experimental diets were formulated as commercial-style starter diets and were cold pelleted as a medium crumble, with the addition of 50 g/kg water, through a 4 mm diameter die. The diets were allowed to cool and dry and were then bagged prior to use.

Experiment 1

The birds were fed one of three commercially formulated dietary treatments based on rice or wheat (Table 34). The wheat diets were based on either a commercial blend or a grain deemed to potentially provide for poor AME. There were six pens per treatment. From 6 to 19 d fresh excreta, and where possible caecal evacuation, pH, was measured. After measurement of pH was completed, a score was applied to the presence or absence of blood-stained mucus in any excreta on the collection tray under each group of birds. At 21 d, 2 birds per pen were killed by cervical dislocation and fresh caecal content samples were collected for pH determination and a separate sample placed immediately on ice. Approximately 1 cm sections of the proximal colo-rectum and distal ileum, 3 cm above the ileo-caecal junction, were excised, flushed with chilled phosphate-buffered saline, and immediately placed in formol-saline. Additional tissue samples were taken from 6 birds; two from each treatment, and sent for histopathological examination by NSW Agriculture, Menangle, NSW. The chilled caecal contents were prepared for VFA and D- and L-lactic acid analyses as per methods described by Taylor (2002).

Experiment 2

The birds were fed one of three commercially formulated dietary treatments based on rice, sorghum or a commercial-blend wheat (Table 34). Each diet was made with or without the addition of virginiamycin (20 mg kg^{-1}). There were three pens per treatment. From 6 to 21d fresh excreta and caecal evacuation pH and scoring of blood-stained mucus in the excreta was performed as above. After the above measures were made at 21 d, 2 birds per pen were killed by cervical dislocation and fresh distal-ileal, caecal and colonic content samples were collected for pH determination and a separate sample of distal-ileal digesta placed immediately on ice. Approximately 1 cm sections of the proximal colo-rectum and distal ileum, 3 cm above the ileo-caecal junction, were excised, flushed with chilled phosphate-buffered saline, and immediately placed in

formol-saline. The chilled ileal digesta were prepared for VFA and D- and L-lactic acid analyses as described by Taylor (2001).

Table 34. Experimental broiler starter diets (g/kg).

	Wheat	Sorghum	Rice
Wheat (120 g/kg CP)	653.8		
Sorghum (90g/kg CP)		625.3	
Rice (80g/kg CP)			578.3
Soybean meal (475 g/kg CP)	229.0	248.0	230.0
Meat meal (520 g/kg CP)	81.0	103.0	131.0
Vegetable oil	18.0	5.6	44.0
Lysine HCl	4.0	3.9	3.3
DL-Methionine	3.3	3.9	3.9
L-Threonine	1.5	1.2	1.5
Salt	0.5		0.3
Sodium bicarbonate	3.4	3.5	2.3
Choline chloride	0.5	0.6	0.4
Vitamin/mineral premix[1]	5.0	5.0	5.0
Calculated specifications			
Metabolisable energy (MJ ME/kg)	12.25	12.25	12.25
Crude Protein (g/kg)	236	234	230
Crude fibre (g/kg)	30	30	20
Lysine (g/kg)	14.5	14.5	14.5
Methionine + Cystine (g/kg)	10.5	10.4	10.5
Fat (g/kg)	44.0	40.1	65.8
Na	2.0	2.0	2.0

[1] The active ingredients (mg/kg) contained in the vitamin and mineral premix are commercial in confidence.

Experiment 3

The birds were fed the commercial broiler starter diet until 20 d of age. From 21 to 29 d either the commercial starter or a commercially formulated diet based on the HP S1 wheat (Table 34) were fed. The wheat diet was made with or without the addition of avilamycin (100 mg kg^{-1}). There were four pens per treatment. From 21d fresh excreta pH and scoring of blood-stained mucus in the excreta was performed daily at the same time. After the above measures were made at 29 d, 2 birds per pen were bled from a jugular vein, killed by cervical dislocation and fresh distal-ileal and caecal digesta samples were collected and placed immediately on ice. Approximately 2 cm sections of the distal ileum, 10 cm above the ileo-caecal junction, were excised and placed immediately in Bouin's solution. After 1 hr the tissues were then placed in 70% ethanol and sent for commercial histopathological examination. The ileal digesta were prepared for D- and L-lactic acid analyses as described by Taylor (2001).

6.3 Results

Experiment 1

Fresh excreta pH was influenced ($p < 0.05$) by the initial pH reading. Excreta pH altered ($p < 0.05$) with diet time (Table 35). Simply explained, the birds on the "high" AME wheat diet produced a lower ($p < 0.05$) excreta pH than birds on the other diets in the last two days. The "high" and "low" AME wheat diets both led to a marked decline in excreta pH compared with the Rice diet and this decline occurred earlier in the trial on the "low" AME wheat than the "high" AME wheat.

Fresh caecal evacuation pH was tested at 18-20 d as little was collected for measurement on a consistent basis prior to these ages. The pH did not alter greatly over the three days (Table 36) and caecal evacuations had a lower ($p < 0.05$) mean pH from birds fed the "high" AME wheat than those fed either the rice or "low" AME wheat diets (6.78 a, 6.52 b and 6.77 a ± 0.073 for the rice, "high" and "low" wheat diets respectively). After slaughter at 21 d the caecal digesta pH (Table 37) provided a similar pattern to that determined from the mean caecal evacuation pH but no significant differences were ($p > 0.05$) discernable. Several birds held little caecal digesta.

Table 35. Mean fresh excreta pH of broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age.

Diet	Age (d)								
	6 (Time0)	8	9	11	13	15	18	19	20
Rice	6.60	6.86 abcd	6.72 bcdef	7.01 ab	6.94 abc	6.96 ab	6.81 bcde	6.93 abcd	6.97 ab
"high" AME wheat	7.10	7.23 a	7.05 ab	6.90 abcd	6.83 bcd	6.85 bcd	6.38 f	6.48 ef	6.45 ef
"low" AME wheat	6.70	6.66 bcdef	6.54 def	6.58 cdef	6.66 bcdef	6.90 abcd	6.68 bcdef	6.98 ab	6.94 abc
SE					0.143				

Table 36. Mean fresh caecal evacuation pH of broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age.

Feed	Age (d)		
	18	19	20
Rice	6.60	6.86	6.88
"high" AME wheat	6.48	6.60	6.48
"low" AME wheat	6.78	6.85	6.68
SE		0.126	

Table 37. Caecal digesta pH of 21 d old broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age.

	Diet (d)		
	Rice	"high" AME wheat	"low" AME wheat
pH	6.84	6.64	6.72
SE	0.125	0.109	0.125
N	10	13	10

Plasma L-lactic acid (Table 38) was higher ($p < 0.05$) in birds fed rice and "high" AME wheat diets than the "low" AME wheat. Ileal D-lactic acid was higher ($p < 0.05$) in birds fed the "high" AME wheat diet than the rice or "low" AME wheat.

Few significant relationships were found between lactic acid concentrations in the plasma and ileal and caecal digesta (Table 39). Ileal and caecal D- and L-lactic acid were found to be positively related ($p < 0.05$). However, exception was found in birds fed the "high" AME wheat where caecal D- and L-lactic acid were not related ($p > 0.05$).

Table 38. Plasma and ileal and caecal digesta L- and D-lactic acid concentration (mMol) of 21 day old broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age.

Sample	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	P
Plasma	Rice	4.30 [a]	0.229	**0.01**	0.005	0.0019	0.23

	"high" wheat	3.92 [a]			0.001		
	"low" wheat	2.95 [b]			0.001		
Ileum	Rice	1.58	0.431	0.42	0.222 [b]	0.1127	**0.04**
	"high" wheat	2.34	0.478		0.625 [a]	0.1250	
	"low" wheat	1.59	0.460		0.255 [b]	0.1205	
Caeca	Rice	0.19	0.097	0.33	0.174	0.0463	0.13
	"high" wheat	0.40	0.100		0.214	0.0479	
	"low" wheat	0.32	0.097		0.307	0.0463	

Table 39. Regression matrix indicating relationships between plasma and ileal and caecal digesta D- and L-lactic acid concentration (mMol) of 21 day old broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age. Probabilities and adjusted R^2 values.

	Plasma D-lactate	Ileal L-lactate	Ileal D-lactate	Caecal L-lactate	Caecal D-lactate
Plasma L-lactate	0.11 0.033	0.30 0.002	0.42 -0.008	0.38 -0.005	0.26 0.007
Plasma D-lactate		0.80 -0.023	0.98 -0.024	0.79 -0.022	0.69 -0.020
Ileal L-lactate			**0.001** **0.785**	0.23 0.013	0.51 -0.015
Ileal D-lactate				0.36 -0.004	0.74 -0.023
Caecal L-lactate					**0.01** **0.129**

No blood was noted in excreta on the day (6 days old) of diet presentation. Scoring of excreta blood (Table 40) commenced at 11 days of age (i.e. 5 d after the trial diets were presented) and a time x diet effect ($p < 0.05$) showed that the rice diet caused greater ($p < 0.05$) fresh blood loss soon after diet presentation, while the response to both wheat diets was delayed but increased rapidly in the last 10 days of the trial. The "low" AME wheat stimulated blood loss quicker than the "high" AME wheat.

Table 40. Scores of blood in excreta (probability ± SE of the probability) of broilers fed rice, "high" or "low" AME wheat starter diets from 6 – 21 days of age.

Feed	Age (d)					
	11	13	15	18	19	20
Rice	0.76 ± 0.127	0.74 ± 0.096	0.71 ± 0.074	0.67 ± 0.072	0.64 ± 0.090	0.60 ± 0.122
"high" AME wheat	0.03 ± 0.273	0.05 ± 0.207	0.11 ± 0.147	0.22 ± 0.103	0.38 ± 0.099	0.58 ± 0.132
"low" AME wheat	0.32 ± 0.132	0.41 ± 0.096	0.52 ± 0.074	0.62 ± 0.075	0.71 ± 0.097	0.79 ± 0.126

Neither SCFA concentrations nor proportions (Table 41) were altered by the dietary cereal.

Table 41. Influence of feeding rice, "high" or "low" AME wheat based diets on the short chain fatty acid (C2-C7) concentration (mMol/L) and short chain fatty acid proportion (%) of total short chain fatty acid (C2-C7) of digesta content in the caeca of 21-d-old male broiler chickens.

Diet	Short chain fatty acid (C2 – C7)								
	acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Rice	48.40	2.60	10.88	1.04	1.02	0.33	0.11	0.10	64.48
"high" AME wheat	37.95	2.78	8.12	1.00	0.65	0.22	0.04	0.04	50.80
"low" AME wheat	40.62	2.33	10.26	0.89	0.70	0.13	0.01	0.02	54.97
SE	3.602	0.361	1.817	0.116	0.190	0.061	0.048	0.045	5.033
Rice	0.756	0.041	0.163	0.016	0.016	0.005	0.0015	0.0014	N/A
"high" AME wheat	0.765	0.058	0.137	0.021	0.012	0.005	0.0010	0.0010	
"low" AME wheat	0.744	0.044	0.180	0.016	0.013	0.003	0.0002	0.0003	
SE	0.0253	0.0059	0.0247	0.0024	0.0030	0.0011	0.00081	0.00079	

Commercial histopathology conducted on distal ileal and proximal colorectal samples indicated that no coccidial oocysts were present, villi were normal, mononuclear cells were as expected, therefore no histological diagnosis was possible. However, comment was made of congestion of superficial colorectal mucosae.

Proportions of fatty acid methyl esters (Table 42) in the ileal mucosa were greater ($p < 0.05$) on the rice than either wheat for C14:0, C16:0 and C16:1n-7 but lower on the rice for C18:2n-6. C18:1n-9 was greater ($p < 0.05$) on the rice and "low AME" wheat than the "high AME" wheat. C14:1n-7 was greater ($p < 0.05$) on the rice than the "low AME" wheat with intermediate proportions on the "high AME" wheat. C20:1n-9 was lower on the rice than the "low AME" wheat with an intermediate proportion for the "high AME" wheat. C22:6n-3 was lower on the rice and "low AME" wheat then the "high AME" wheat. Differences approached significance for four other fatty acids as indicated in Table 42.

Table 42. Fatty acid methyl esters (% total fatty acid) in the ileal mucosa of broilers fed rice, "high AME" or "low AME" wheat-based diets from 6-21 of age.

Fatty acid	Cereal			SE	
	Rice [a]	High AME Wheat [a]	Low AME wheat [b]	n=6 [a]	n=5 [b]
C8:0	0	0	0	0	0
C10:0	0.02	0.01	0	0.007	0
C14:0	0.74 [a]	0.48 [b]	0.51 [b]	0.049	0.054
C14:1n-7	0.18 [a]	0.16 [ab]	0.08 [b]	0.024	0.026
C16:0	23.36 [a]	20.77 [b]	20.39 [b]	0.769	0.842
C16:1n-7	6.68 [a]	3.54 [b]	4.60 [b]	0.499	0.546
C18:0	11.38	13.96	13.01	0.833	0.912
C18:1n-9	39.15 [a]	31.21 [b]	36.39 [a]	1.589	1.740
C18:1n-7 *	2.99	3.03	3.32	0.097	0.106
C18:2n-6	9.64 [b]	15.80 [a]	13.74 [a]	0.766	0.840
C18:3n-6	0.11	0.16	0.17	0.021	0.023
C18:3n-3 **	0.35	0.52	0.50	0.049	0.054
C20:0	0.47	0.62	0.63	0.079	0.086
C20:1n-9	0.46 [b]	0.50 [ab]	0.54 [a]	0.014	0.015
C20:2n-6	0.33	0.25	0.23	0.070	0.077
C20:3n-6	0.61	0.93	0.80	0.137	0.150
C20:4n-6 ***	2.02	5.05	2.83	0.841	0.922
C20:5n-3	0.29	0.48	0.36	0.064	0.070
C22:0	0.19	0.37	0.30	0.068	0.075
C22:1n-9	0.10	0.07	0.12	0.047	0.051
C22:2n-6	0.11	0.19	0.17	0.041	0.045
C22:3n-3	0.23	0.26	0.31	0.058	0.063
C22:5n-3 ****	0.21	0.45	0.32	0.069	0.075
C22:6n-3	0.28 [b]	0.97 [a]	0.47 [b]	0.148	0.162
C24:0	0.03	0.12	0.11	0.040	0.044
C24:1n-9	0.06	0.10	0.10	0.041	0.045

*p=0.080; **p=0.055; ***p=0.061; ****p=0.090

Experiment 2

The excreta pH was not altered ($p > 0.05$) over time by the grain base of the diet. Virginiamycin (VM) use maintained a generally higher ($p < 0.05$) excreta pH than unsupplemented diets over time (Table 43). A grain x virginiamycin interaction (Table 44) was found with rice minus VM producing the lowest ($p < 0.05$) mean excreta pH, the VM supplemented rice producing a higher ($p < 0.05$) excreta pH similar ($P > 0.05$) to the VM supplemented wheat, wheat producing similar pH irrespective of VM use and the sorghum producing the highest ($p < 0.05$) excreta pH with no influence ($p > 0.05$) of VM.

Fresh caecal evacuation pH, again only tested at 18, 19 and 20 d, only produced differences across the three cereals with higher ($p < 0.05$) pH produced by the sorghum diet than the rice or wheat diets (6.77, 7.17 and 6.81 ± 0.123, for rice, sorghum and wheat respectively).

Table 43. Excreta pH of broilers fed Virginiamycin (VM) unsupplemented or supplemented diets from 1 – 21 days of age.

Diet	Age (d)								
	6	8	9	11	13	15	18	19	20
Minus VM	7.00	7.18	7.42	7.59	7.38	7.50	7.28	7.51	7.32
	a	ab	bcd	def	bcd	cdef	bc	cdef	bc
Plus VM	7.22	7.51	7.43	7.31	7.61	7.66	7.77	7.71	7.69
	ab	cdef	bcde	bc	def	ef	f	f	ef
SE					0.095				

Table 44. Mean excreta pH of broilers fed rice, sorghum or wheat diets plus or minus Virginiamycin (VM) from 1-21 days of age.

Diet	Grain		
	Rice	Sorghum	Wheat
Minus VM	6.84 a	8.14 d	7.08 b
Plus VM	7.24 c	8.20 d	7.20 bc
SE		0.055	

After slaughter at 21 d the ileal and colonic digesta pH (Table 45) was not altered ($p > 0.05$) by cereal. A cereal x VM interaction produced similar ($p > 0.05$) and low caecal digesta pH on the wheat diet, higher ($p < 0.05$) pH on the rice diet, irrespective of VM use, and a high pH on the sorghum diet minus VM. With addition of VM to the sorghum diet, caecal digesta pH was reduced ($p < 0.05$) and similar to that of the wheat diets.

Table 45. Ileal, caecal and colonic digesta pH of 21 d old broilers fed rice, sorghum or wheat diets plus or minus Virginiamycin (VM) from 1-21 days of age.

Gut section	VM	Grain		
		Rice	Sorghum	Wheat
Ileum	Minus	8.58	8.50	8.18
	Plus	8.18	8.48	8.28
SE		0.240	0.240	0.215
Caeca	Minus	6.15 b	6.54 a	5.74 c
	Plus	6.47 ab	5.65 c	5.78 c
SE		0.124	0.136	0.136
Colon	Minus	7.52	7.66	7.40
	Plus	7.13	7.98	7.63
SE		0.234	0.256	0.286

Plasma D- & L- and ileal L-lactic acid (Table 46) were not altered ($p > 0.05$) by cereal or VM application. Ileal D-lactic acid was higher ($p < 0.05$) in birds fed the sorghum and wheat diets without VM than in those fed rice minus VM or wheat plus VM. Rice and sorghum plus VM diets produced ileal digesta pH's intermediate between these two groups.

Table 46. Plasma and ileal digesta L- and D-lactic acid concentration (mMol) of 21 day old broilers fed rice, sorghum or wheat diets plus or minus Virginiamycin (VM) from 1-21 d.

Sample	Feed	VM	L-lactic acid			D-lactic acid		
			LS Mean	SE	P	LS Mean	SE	P
Plasma	Rice	–	3.42	0.348	0.20	0.005	0.0034	0.88
	Rice	+	2.36	0.381		0.006	0.0037	
	Sorghum	–	2.78	0.348		0.008	0.0034	
	Sorghum	+	2.43	0.348		0.007	0.0034	
	Wheat	–	2.67	0.348		0.003	0.0034	
	Wheat	+	2.20	0.348		0.003	0.0034	
Ileum	Rice	–	0.71	0.176	0.98	0.022 b	0.0219	**0.04**
	Rice	+	0.76	0.176		0.068 ab	0.0219	
	Sorghum	–	0.67	0.161		0.093 a	0.0200	

Sorghum	+	0.70	0.197	0.055 ab	0.0245
Wheat	–	0.58	0.161	0.087 a	0.0200
Wheat	+	0.65	0.161	0.013 b	0.0200

One significant ($p < 0.05$) relationship, between D- and L-lactic acid concentrations in the plasma, was found. Plasma and ileal lactic acid concentrations were not related ($p > 0.05$).

As noted in the previous experiment, no blood was noted in excreta before the diets were fed to the chicks. The cereal did not alter ($p > 0.05$) blood scores but VM use reduced blood loss scores over time ($p < 0.05$) (Table 47).

Table 47. Scores of blood in excreta (probability ± SE of the probability) of broilers fed rice, sorghum or wheat diets plus or minus Virginiamycin (VM) from 1-21 days of age.

Feed	Age (d)					
	11	13	15	18	19	20
Rice	0.26 ± 0.172	0.23 ± 0.129	0.20 ± 0.106	0.18 ± 0.115	0.16 ± 0.153	0.14 ± 0.203
Sorghum	0.11 ± 0.229	0.12 ± 0.173	0.13 ± 0.131	0.14 ± 0.121	0.16 ± 0.149	0.18 ± 0.199
Wheat	0.33 ± 0.156	0.31 ± 0.117	0.29 ± 0.094	0.27 ± 0.097	0.25 ± 0.127	0.23 ± 0.169
Minus VM	0.13 ± 0.164	0.16 ± 0.123	0.20 ± 0.092	0.23 ± 0.082	0.28 ± 0.098	0.33 ± 0.131
Plus VM	0.35 ± 0.133	0.27 ± 0.100	0.19 ± 0.090	0.14 ± 0.112	0.10 ± 0.154	0.07 ± 0.203

Neither SCFA concentrations nor proportions (Table 48) were altered by the dietary cereal or virginiamycin use.

Table 48. **Influence of feeding rice, sorghum or wheat minus or plus VM on the short chain fatty acid (C2-C7) concentration (mMol/L) and short chain fatty acid proportion (%) of total short chain fatty acid (C2-C7) of digesta content in the ileum of 21-d-old male broiler chickens.**

Cereal	VM	Short chain fatty acid (C2 – C7)								
		acetic	Propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Rice	–	9.25	0.16	0.73	0.90	0.15	0	0.01	0.03	11.22
Rice	+	9.46	0.03	0.12	1.21	0.07	0.04	0.01	0.03	10.97
Sorghum	–	6.50	0.16	0.34	1.09	0.08	0.02	0.03	0.02	8.23
Sorghum	+	5.10	0.10	0.18	1.00	0.05	0	0	0.01	6.44
Wheat	–	6.49	0.06	0.16	1.07	0.03	0.01	0.01	0.01	7.84
Wheat	+	7.07	0.01	0.08	1.08	0.07	0	0.02	0.03	8.36
SE		1.380	0.058	0.197	0.186	0.026	0.011	0.009	0.011	1.573
Rice	–	0.828	0.012	0.058	0.085	0.013	0	0.001	0.003	N/A
Rice	+	0.856	0.004	0.011	0.115	0.007	0.004	0.001	0.003	
Sorghum	–	0.821	0.015	0.032	0.117	0.008	0.002	0.003	0.002	
Sorghum	+	0.805	0.011	0.021	0.156	0.006	0	0	0.001	
Wheat	–	0.801	0.004	0.014	0.175	0.004	0.001	0.001	0.001	
Wheat	+	0.832	0.001	0.001	0.149	0.007	0	0.002	0.003	
SE		0.0276	0.0049	0.0163	0.0229	0.0026	0.0009	0.0010	0.0011	

Experiment 3

Excreta pH altered ($p < 0.05$) with diet over time (Table 49). Periods of very hot weather appeared to be associated with diarrhoea and so may have contributed to pH changes in birds on the commercial diet; excreta pH fluctuated. These fluctuations were moderated on the wheat diets which, irrespective of avilamycin incorporation, tended to maintain higher pH of excreta, especially over the last three days of the experiment.

Table 49. Mean fresh excreta pH of broilers fed a commercial starter diet or a wheat (HP S1) –based diet with or without avilamycin from 21 – 29 days of age.

Diet	Age (d)								
	21 (Time0)	22	23	24	25	26	27	28	29
Commercial	7.23 def	6.51 g	7.18 def	7.49 abcd	7.80 abc	7.57 abcd	6.93 fg	6.98 efg	6.60 g
HP S1 wheat	7.23 def	7.39 cdef	7.24 def	7.25 def	7.38 cdef	7.61 abcd	7.42 bcde	7.88 ab	7.49 abcd
HP S1 wheat + avilamycin	7.19 def	7.39 cdef	7.26 def	7.94 a	7.51 abcd	7.58 abcd	7.79 abc	7.61 abcd	7.62 abcd
SE					0.245				

Ileal L-lactic acid (Table 50) was higher ($p < 0.05$) in birds fed the wheat diet irrespective of avilamycin use. Plasma and caecal L- and D-lactic acid was not influenced ($p > 0.05$) by diet. Ileal D-lactic acid displayed a similar trend ($p = 0.059$) to L-lactic acid.

Table 50. Plasma and ileal and caecal digesta L- and D-lactic acid concentration (mMol/l) of 29 day old broilers fed a commercial or an HP S1 wheat diet with or without avilamycin from 21 – 29 days of age.

Sample	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Plasma	Commercial	5.17	0.423	0.78	0.03	0.121	0.11
	HP S1 wheat	5.49			0.40		
	HP S1 + avilamycin	5.09			0.17		
Ileum	Commercial	3.35 [b]	5.558	**0.01**	1.79	4.186	0.06*
	HP S1 wheat	24.78 [a]	5.322		14.27	4.008	
	HP S1 + avilamycin	28.91 [a]	5.558		14.72	4.186	
Caeca	Commercial	1.99	0.315	0.11	2.26	0.348	0.10
	HP S1 wheat	1.02	0.315		1.18	0.348	
	HP S1 + avilamycin	1.51	0.329		1.55	0.363	

* p value approaching 0.05.

Plasma D-lactic acid levels increased ($p < 0.05$) with increasing ileal L- and D-lactic acid levels (Table 51). Conversely, plasma L-lactic acid levels were unrelated ($p > 0.05$) to either lactic acid isomer in gut sections. Ileal and caecal D- and L-lactic acid were positively related ($p < 0.05$).

Some few blood scores of excreta were positive the day before and day of diet presentation (days 20 and 21) (Table 52). The wheat diets produced more blood loss ($p < 0.05$) than the commercial diet over the full experimental period. Inclusion of avilamycin in the HP S1 wheat diet did not affect ($p > 0.05$) excreta blood loss.

Table 51. Regression matrix indicating relationships between plasma and ileal and caecal digesta D- and L-lactic acid concentration (mMol/l) of 29 day old broilers fed a commercial diet or a diet with or without avilamycin inclusion from 21 – 29 days of age. Figures are probabilities and adjusted R^2 values of the linear regression equations.

	Plasma D-lactate	Ileal L-lactate	Ileal D-lactate	Caecal L-lactate	Caecal D-lactate
Plasma L-lactate	0.49 -0.016	0.65 -0.025	0.36 -0.005	0.96 -0.032	0.88 -0.031
Plasma D-lactate		**0.001** **0.25**	**0.001** **0.50**	0.36 -0.004	0.30 0.003
Ileal L-lactate			**0.001** **0.85**	0.64 -0.025	0.43 -0.011
Ileal D-lactate				0.43 -0.011	0.28 0.007
Caecal L-lactate					**0.97** **0.001**

Table 52. Scores of blood in excreta (probability ± SE of the probability) of broilers fed a commercial diet or a diet with or without avilamycin inclusion from 21 – 29 days of age.

Feed	Age (d)									
	20	21 (time0)	22	23	24	25	26	27	28	29
Commercial	0.02 0.304	0.02 0.263	0.03 0.223	0.03 0.185	0.04 0.152	0.06 0.127	0.07 0.116	0.09 0.121	0.12 0.142	0.15 0.172
HP S1 wheat	0.06 0.169	0.08 0.145	0.11 0.121	0.15 0.099	0.21 0.081	0.28 0.069	0.36 0.067	0.45 0.074	0.54 0.087	0.64 0.103
HP S1 + avilamycin	0.08 0.153	0.11 0.130	0.14 0.109	0.19 0.089	0.25 0.074	0.31 0.065	0.39 0.065	0.47 0.073	0.55 0.086	0.63 0.101

Diarrhoea was incurred on the two wheat diets irrespective of avilamycin use (Table 53). These diarrhoea data approached significance ($p = 0.103$). With the hot weather some wet excreta were produced by some birds from time to time but this became consistent from most of the broilers in each of the pens fed the wheat diets within 24 h of presentation of the feed. The diarrhoea present was not simply moist excreta but watery, loose excreta that wet most of the excreta tray surface. Mucus appeared throughout these wet excreta.

Table 53. Scores of diarrhoea (probability ± SE of the probability) from of broilers fed a commercial diet or a diet with or without avilamycin inclusion from 21 – 29 days of age.

Feed	Age (d)									
	20	21 (time0)	22	23	24	25	26	27	28	29
Commercial	0.03 0.437	0.03 0.368	0.03 0.310	0.03 0.268	0.03 0.252	0.02 0.267	0.02 0.307	0.02 0.365	0.02 0.433	0.02 0.508
HP S1 wheat	0.21 0.174	0.30 0.140	0.42 0.111	0.54 0.093	0.66 0.091	0.76 0.102	0.84 0.121	0.89 0.145	0.93 0172	0.96 0.201
HP S1 + avilamycin	0.16 0.187	0.26 0.149	0.38 0.117	0.53 0.098	0.67 0.096	0.78 0.111	0.87 0.134	0.92 0.162	0.95 0.193	0.97 0.225

Plasma myeloperoxidase analysis provided data below detection limits. Commercial histopathology conducted on distal ileal tissue samples indicated a range of cellular infiltrates, lymphoid foci with or without lymphoid infiltrate into the coria and/or germinal centres in the propria. There were substantial differences between individual broilers leading to no consistent treatment variation being discernible.

6.4 Discussion

Excreta pH changes were noted with a change in cereal inclusion in the first experiment where the birds were changed from their standard commercial feed at 5 d. The differences in components of the three grains may have been responsible for the changes in pH over the 16 d of the experiment. Rice contains little structural carbohydrates, NSP content being minimal and is considered highly fermentable. The effects of the rice and "low" AME wheat appeared to commence earlier than from the "high" AME wheat and pH changes had resolved before those of the "high" AME wheat. The excreta blood losses were of a similar pattern with immediate increases in blood lost from birds on the rice diet, followed by the "low" AME wheat and, over the final few days, blood loss occurred from birds on the "high" AME wheat. At

this time, on the "high" AME wheat, lactic acid contents in the ileal digesta may have been higher as suggested by the greater D-lactic acid concentration.

The considerable variation in histopathology across individual birds provided for no discernable differences across cereal types but, as each grain produced blood loss in the excreta, the general conclusion of colo-rectal congestion across samples suggests that all feeds created some inflammation of the hindgut mucosae. The differences in the ileal mucosa fatty acid profile indicates the effect of diet upon tissues and suggests that diet must be considered for its alteration of membrane phospholipids in the gastro-intestinal tract with effects on disease conditions like ulcerative colitis and gastritis as well as of nutrient absorption by the enterocyte (Garg *et al*., 1997). The gut is active in synthesizing eicosanoids (prostaglandins and leukotrienes) using 20:4 n-6 as a substrate, and this increases in ulcerative colitis and other inflammatory conditions (Garg *et al*., 1997). The current results suggested a lower C20:4 n-6 content in the ileal mucosa of birds on the rice and "low" AME wheat diets; those that had produced much early blood loss upon dietary change.

Feeding different cereals from day old had a much more subtle effect on the birds to 21 d. There were differences in the overall mean excreta pH with sorghum maintaining a higher pH than either rice or wheat. This result confirms a similar and consistent finding of earlier work (Taylor, 2002). The predominant result of virginiamycin inclusion was that blood loss occurred in some birds (a single appearance of blood resulted in a score of "presence" under a group cage) despite a consistent diet being presented to the birds. However, without virginiamycin in the feed, the appearance of blood in excreta increased over the 21 d of the experiment. Inclusion of virginiamycin was associated with more early blood loss but minimal loss by the final 5-6 d. Chicks have complex gut microbial populations at hatch but the populations change, especially over the first three d (Barnes *et al*., 1980) with some, such as non-sporing anaerobes only becoming established at ~21 d (Barnes *et al*., 1979). Lee (2002) indicated that virginiamycin use resulted in gross alteration of the ratio of Clostridia:Lactobacilli and the current results may reflect some effects of these different groups on the hindgut in the starter period in broilers.

In older birds, the use of a "problem" wheat certainly caused much blood loss in the excreta and most birds suffering diarrhoea despite maintenance of a stable excreta pH. Lactic acid concentrations were increased, both L- and D-isomers above the levels of lactic acidosis in the sheep intestine (Ding and Xu, 2003) and a relationship between plasma and ileal D- and L-lactic acids was established under these conditions. As these older birds may be assumed to have had well established microbial populations in the hindgut, the lack of response to avilamycin was of note.

Consideration of the effects of viable microbial populations adherent to/associated with the various cereals led to another series of trials.

7. Effects of dietary cereal change on gut pH, fermentation and tissue in SPF Leghorns.

7.1 Introduction

A major problem with many nutrition trials aimed at dealing with effects of feeds and interactions with additives such as exogenous enzymes and antibiotics is that the birds and/or feeds employed have disease risks and/or microbial populations that compromise the results. Birds may have been compromised before an experiment commences; feeds may carry micro-organism loads of unknown potential; ordinary micro-organism loads may create the possibility of altering established gut organism populations. Although this could be excused as reflecting the normal field situation, for the purposes of these experiments it would seem imperative to remove as many complicating factors as possible to determine the influences of a simple change in the dietary cereal base on hindgut function in the bird.

The use of specific pathogen-free (SPF) birds and feeds devoid of an active micro-organism population would normally be precluded in nutrition trials because of the expense involved in procuring and maintaining facilities and birds and of feed sterilisation.

The opportunity to use such birds to develop the results obtained in earlier work (Taylor, 2002) arose and enabled development of further work with disease free birds and using various combinations of feed types after the destruction of their viable microbial populations.

7.2 Materials and methods

The birds were SPF Leghorn-derived lines (SWL, HWL or a hybrid (cross) maintained for commercial vaccine manufacture and testing. The birds were hatched from SPF eggs and grown from day old, in filtered-air, positive-pressure (FAPP) isolators. Feed and water were provided through either aseptic or sterile procedures to the birds. Feeds were sterilized either by gas (ethylene oxide) or gamma irradiation (50 kG kg^{-1} imparted energy).

Half of each group of birds, on commercial diets (control) in FAPP isolators, were removed by sterile procedure, killed by cervical dislocation and gut organ/section digesta pH measured. The remaining birds were fed on sterilised, different cereal-based feeds for three days then killed and treated as above. Digesta samples were taken from both groups of birds and placed on ice for determination of volatile fatty acid and lactate concentrations as described by Taylor (2001).

Experiments

Trial 1a:	Ethylene oxide sterilised commercial starter feed. Birds 21 then 56 d old.
Trial 1b:	Ethylene oxide sterilised commercial starter feed and irradiated “low AME” wheat for two days. Birds 57 and 59 d old.
Trial 2:	Birds brooded together then grown in two FAPP isolators. Irradiated commercial starter and irradiated Weston commercial wheat diet either whole or ground wheat + enzyme (Allzyme PT 1 g kg^{-1}) for two days: commercial and whole wheat diet in one isolator; commercial and ground wheat diet in the other. Birds 56 and 58 d old.
Trial 3:	Irradiated commercial starter and irradiated Weston commercial ground wheat diet + Virginiamycin (20 mg kg-1) for two days. Birds 57 and 59 d old.

Trial 4: Unsterile commercial starter and irradiated ground rice diet for two days. Birds 56 and 58 d old.

Trial 5: Unsterile commercial starter and grower then birds fed on irradiated wheat, rice and barley, ground-cereal cold-pelleted diets for four days. Birds 59 and 62 d old.

Trial 6: Unsterile commercial starter and grower then birds fed on irradiated wheat, rice and barley, whole-cereal cold-pelleted diets for four days. Birds 69 and 73 d old.

7.3 Results

Feed intake was not measurable but changes of diets did not cause noticeable alteration to total feed consumption or time for feed to be depleted. This reflected the lack of feed intake response to diet change noted in all the open-house trials conducted to date.

The methods employed were similar over each experiment but the earlier experiments were used to develop consistency and rapidity. In fact, many extra individual trials were conducted to develop and settle upon both methods and feed types i.e. 3 test trials with various feeds (unsterile, gas sterilised or irradiated feeds) were conducted prior to the series reported.

Male body weight was greater ($p < 0.05$) than females as expected. Some interactions of diet and sex were observed but are considered, largely, an artefact of the numbers of birds available (experimental number of males remaining to be fed the test diets was often minimal) as sex could not be determined either in the hatching isolators or prior to placement of d old chicks in the test isolators. The numbers of chicks placed for routine vaccine testing had been determined by long experience to allow for reasonable numbers of either sex. Body weight is presented in tables for completeness.

Trial 1a

Digesta pH (Table 54) was lower ($p < 0.05$) in the caecum of the older birds (6.64a ± 0.115 and 6.04b ± 0.103 at 21 and 56 d, respectively). Gizzard and ileal pH was unchanged ($p > 0.05$) from 21 d to 56 d of age.

Table 54. Influence of age on digesta pH of SPF leghorns at 21 and 56 d fed continuously on an ethylene oxide sterilised commercial starter diet.

Gut section	Age (d)	Sex	LS Mean	SE (LS mean)	P
Gizzard	21	♀	2.90	0.083	0.516
	21	♂	2.98	0.102	
	56	♀	3.01	0.083	
	56	♂	3.08	0.083	
Ileum	21	♀	7.70	0.145	0.224
	21	♂	7.38	0.154	
	56	♀	8.01	0.131	
	56	♂	7.75	0.154	
Caecum	21	♀	6.64	0.146	**0.004**
	21	♂	6.63	0.178	
	56	♀	5.94	0.146	
	56	♂	6.13	0.146	

Trial 1b

Digesta pH (Table 55) was higher ($p < 0.05$) in the crop (5.85a and 5.33b ± 0.106) and gizzard (3.44a and 2.69b ± 0.106) but lower ($p < 0.05$) in the ileum (7.54b and 7.88a ± 0.106) of birds fed the commercial rather than the "low AME" feed. Ileal digesta pH was not affected ($p > 0.05$) by diet. Sex did not influence ($p > 0.05$) digesta pH. Male body weight (Table 55) was greater ($p < 0.05$) than the female but was not influenced ($p > 0.05$) by diet.

Table 55. Influence of ethylene oxide sterilised commercial starter or irradiated "low AME" wheat diets on the digesta pH and body weight of SPF leghorns at 57 and 59 d old.

Gut section	Feed	Sex	LS Mean	SE (LS mean)	P
Crop	Commercial	♀	5.78	0.180	**0.047**
	Commercial	♂	5.93	0.191	
	"low AME" wheat	♀	5.46	0.163	
	"low AME" wheat	♂	5.20	0.191	
Gizzard	Commercial	♀	3.56	0.128	**0.001**
	Commercial	♂	3.33	0.136	
	"low AME" wheat	♀	2.72	0.116	
	"low AME" wheat	♂	2.66	0.136	
Ileum	Commercial	♀	7.70	0.145	**0.032**
	Commercial	♂	7.38	0.154	
	"low AME" wheat	♀	8.01	0.131	
	"low AME" wheat	♂	7.75	0.154	
Caecum	Commercial	♀	6.29	0.091	0.613
	Commercial	♂	6.34	0.097	
	"low AME" wheat	♀	6.45	0.082	
	"low AME" wheat	♂	6.40	0.097	
Body weight (g)	Commercial	♀	530	19.5	**0.041**
	Commercial	♂	591	20.7	
	"low AME" wheat	♀	532	17.6	
	"low AME" wheat	♂	591	20.7	

Trial 2

Digesta pH (Table 56) in the gizzard was not influenced ($P > 0.05$) by isolator, feed or sex. Digesta pH was higher ($p < 0.05$) in the ileum (7.77a, 7.06b and 7.67a ± 0.176) of birds fed the commercial and ground wheat + enzyme diets than those fed the whole wheat diet + enzyme but was not influenced ($p > 0.05$) by isolator or sex. Caecal digesta pH was higher ($p < 0.05$) in isolator 1 (commercial and whole wheat feeds) than isolator 2 (commercial and ground wheat feeds) (6.22a ± 0.071 and 5.96b ± 0.068), higher ($p < 0.05$) in female than male birds (6.20a ± 0.047 and 5.98b ± 0.068) but was not influenced ($p > 0.05$) by diet.

Table 56. Influence of irradiated commercial starter or irradiated Weston commercial whole and ground wheat + enzyme diets on the digesta pH and body weight of SPF leghorns at 56 and 58 d old.

Gut section	Feed	Sex	LS Mean	SE (LS mean)	P
Gizzard	Commercial	♀	3.34	0.137	0.404
	Commercial	♂	3.38	0.208	
	Whole wheat + e	♀	3.59	0.216	
	Whole wheat + e	♂	3.33	0.383	
	Ground wheat + e	♀	3.79	0.266	
	Ground wheat + e	♂	3.02	0.320	
Ileum	Commercial	♀	7.96	0.095	**0.001**
	Commercial	♂	7.57	0.143	Feed
	Whole wheat + e	♀	7.22	0.152	
	Whole wheat + e	♂	6.91	0.293	
	Ground wheat + e	♀	7.63	0.193	
	Ground wheat + e	♂	7.72	0.294	
Caecum	Commercial	♀	6.29	0.062	**0.004**
	Commercial	♂	5.90	0.093	Isolator
	Whole wheat + e	♀	6.08	0.097	Sex
	Whole wheat + e	♂	5.83	0.172	
	Ground wheat + e	♀	6.23	0.119	
	Ground wheat + e	♂	6.23	0.144	
Body weight (g)	Commercial	♀	693	12.1	**0.001**
	Commercial	♂	836	18.3	Isolator
	Whole wheat + e	♀	712	19.0	Sex
	Whole wheat + e	♂	774	33.7	
	Ground wheat + e	♀	670	23.4	
	Ground wheat + e	♂	842	28.2	

Trial 3

Digesta pH (Table 57) in the crop and gizzard was not influenced ($P > 0.05$) by feed or sex. Ileal digesta pH was higher ($p < 0.05$) on the wheat + VM diet and in females (Table 57). Caecal digesta pH was lower ($P < 0.05$) on the wheat + VM diet but unaffected ($P > 0.05$) by sex. Colonic digesta pH was not affected by feed ($P > 0.05$) but was higher ($P < 0.05$) in females.

Table 57. Influence of irradiated commercial starter or irradiated Weston commercial ground wheat + VM diets on the digesta pH and body weight of SPF leghorns at 57 and 59 d old.

Gut section	Feed	Sex	LS Mean	SE (LS mean)	P
Crop	Commercial	♀	5.95	0.239	0.268
	Commercial	♂	5.59	0.207	
	Ground wheat + VM	♀	6.18	0.207	
	Ground wheat + VM	♂	5.98	0.239	
Gizzard	Commercial	♀	3.51	0.203	0.380
	Commercial	♂	3.39	0.217	
	Ground wheat + VM	♀	3.64	0.203	
	Ground wheat + VM	♂	3.93	0.235	
Ileum	Commercial	♀	8.19	0.068	**0.001**
	Commercial	♂	7.79	0.068	Feed
	Ground wheat + VM	♀	8.53	0.068	Sex
	Ground wheat + VM	♂	8.05	0.079	
Caecum	Commercial	♀	6.04	0.077	**0.045**
	Commercial	♂	5.94	0.077	Feed
	Ground wheat + VM	♀	5.75	0.077	

	Ground wheat + VM	♂	5.77	0.089	
Colon	Commercial	♀	6.61	0.273	**0.047**
	Commercial	♂	6.47	0.315	Sex
	Ground wheat + VM	♀	7.43	0.292	
	Ground wheat + VM	♂	6.22	0.315	
Body weight (g)	Commercial	♀	690	29.4	**0.001**
	Commercial	♂	839	29.4	F x S
	Ground wheat + VM	♀	678	29.4	
	Ground wheat + VM	♂	964	34.0	

Trial 4

Digesta pH (Table 58) in the crop, gizzard, jejunum and ileum was not influenced ($P > 0.05$) by feed or sex (ileal digesta pH approached difference across sex). Caecal digesta pH was higher ($p < 0.05$) on the rice diet (Table 58).

Table 58. Influence of unsterile commercial starter or irradiated ground rice diets on the digesta pH and body weight of SPF leghorns at 56 and 58 d old.

Gut section	Feed	Sex	LS Mean	SE (LS mean)	P
Crop	Commercial	♀	5.90	0.233	0.322
	Commercial	♂	5.35	0.251	
	Rice	♀	5.86	0.195	
	Rice	♂	5.92	0.251	
Gizzard	Commercial	♀	4.14	0.154	0.906
	Commercial	♂	4.00	0.154	
	Rice	♀	4.06	0.138	
	Rice	♂	4.14	0.178	
Jejunum	Commercial	♀	7.23	0.112	0.333
	Commercial	♂	7.07	0.112	
	Rice	♀	7.27	0.101	
	Rice	♂	7.01	0.130	
Ileum	Commercial	♀	8.03	0.092	0.051*
	Commercial	♂	7.79	0.092	Sex
	Rice	♀	8.12	0.082	
	Rice	♂	7.86	0.106	
Caecum	Commercial	♀	5.73	0.102	**0.024**
	Commercial	♂	5.43	0.102	Feed
	Rice	♀	5.88	0.092	
	Rice	♂	5.76	0.118	
Body weight (g)	Commercial	♀	633	19.8	**0.001**
	Commercial	♂	773	19.8	Feed
	Rice	♀	683	17.7	Sex
	Rice	♂	816	22.8	

* Ileal digesta pH approached significance.

Trial 5

Digesta pH (Table 59) in the crop was lower ($p < 0.05$) in birds on the wheat diet and in males. Gizzard digesta pH was not influenced ($p > 0.05$) by diet or sex. Jejunal digesta pH was lower ($p < 0.05$) on the wheat diet. Ileal digesta pH was not influenced ($p > 0.05$) by feed but approached significance with sex (lower in males). Caecal digesta pH was lower ($p < 0.05$) on the wheat diet but was not affected ($p > 0.05$) by sex. Digesta pH (Table 59) on the rice diet was not influenced ($p > 0.05$) by diet or sex. Digesta pH (Table 59) did not differ ($p > 0.05$) in any gut organ/section but a trend to lower jejunal and caecal pH approached significance on the barley diet.

Table 59. Influence of unsterile commercial starter or irradiated ground wheat, rice or barley diets on the digesta pH and body weight of SPF leghorns at 59 and 62 d old.

Feed	Sex	n	Crop	Gizzard	Jejunum	Ileum	Caeca	Body wt.
Commercial	♀	11	**6.09** [ax]	4.28	**7.41** [a]	8.48	**6.46** [a]	**655** [d]
Commercial	♂	8	**5.80** [ay]	3.95	**7.23** [a]	7.84	**6.27** [a]	**792** [b]
Wheat	♀	12	**5.45** [bx]	3.74	**6.89** [b]	8.00	**5.92** [b]	**702** [c]
Wheat	♂	7	**5.11** [by]	3.76	**6.70** [b]	7.80	**5.71** [b]	**972** [a]
SE			0.141	0.168	0.147	0.185	0.156	15.9
			0.158	0.197	0.172	0.207	0.183	18.6
			0.095	0.161	0.140	0.169	0.150	15.2
			0.120	0.210	0.184	0.221	0.196	19.9
P			**0.01**	0.12	**0.02**	0.07	**0.02**	**0.01**
Commercial	♀	9	5.23	3.23	6.95	8.40	6.54	**670** [by]
Commercial	♂	6	4.71	3.20	6.56	7.85	6.23	**761** [bx]
Rice	♀	5	5.15	3.47	6.72	8.16	6.52	**732** [ay]
Rice	♂	11	5.18	3.44	7.02	7.87	6.31	**877** [ax]
SE			0.560	0.207	0.129	0.189	0.165	21.3
			0.396	0.239	0.158	0.189	0.202	26.0
			0.250	0.262	0.173	0.267	0.222	28.5
			0.177	0.177	0.137	0.101	0.149	19.2
P			0.74	0.74	0.11	0.15	0.55	**0.01**
Commercial	♀	9	—	3.47	7.14	8.10	6.76	**672** [y]
Commercial	♂	6	—	3.42	7.42	—	6.54	**824** [x]
Barley	♀	8	5.19	3.79	6.58	7.76	6.47	**712** [y]
Barley	♂	8	4.88	3.97	6.40	7.46	6.07	**842** [x]
SE			—	0.186	0.261	0.291	0.164	29.1
			—	0.227	0.350	—	0.201	35.7
			0.140	0.197	0.277	0.110	0.174	30.9
			0.140	0.197	0.277	0.103	0.174	30.9
P			0.15	0.20	0.08*	0.07*	0.06*	**0.01**

Caecal digesta L- or D-lactic acids (Table 60) were influenced ($p > 0.05$) by feed and sex only on the wheat feed and not the rice or barley feeds. However, a feed x sex interaction approached significance on the rice diet. Females fed the wheat diet had higher L-lactate concentrations in the caecal digesta then did males or birds of either sex on the commercial diet. Both sexes incurred higher ($p < 0.05$) D-lactic acid on the wheat diet than did females on the commercial diet but with males on the commercial diet having concentrations between the two.

Table 60. Influence of feeding an unsterile commercial grower then irradiated ground wheat, rice or barley diets to SPF leghorns on the concentration (mMol/L) of L- and D-lactic acid in caecal digesta at 59 and 62 d old.

Feed	Sex	n	L-lactic acid	SE	Factor	p	D-lactic acid	SE	Factor	P
Commercial	♀	9	0.58 [b]	0.142	F	0.02	0.57 [b]	0.138	F	0.02
Commercial	♂	6	0.93 [b]	0.174	S	0.65	0.98 [ab]	0.169	S	0.71
Wheat	♀	10	1.39 [a]	0.134	F x S	**0.01**	1.30 [a]	0.131	F x S	**0.03**
Wheat	♂	7	0.89 [b]	0.161			1.00 [a]	0.156		
Commercial	♀	6	0.92	0.957	F	0.09	0.92	0.779	F	0.27
Commercial	♂	4	4.39	1.172	S	0.13	3.77	0.953	S	0.20
Rice	♀	5	1.07	1.049	F x S	0.07*	1.35	0.853	F x S	0.07*
Rice	♂	10	0.70	0.741			1.09	0.603		
Commercial	♀	5	0.91	0.666	F	0.23	0.99	0.405	F	0.15
Commercial	♂	1	0	1.490	S	0.81	0.60	0.906	S	0.73
Barley	♀	8	0.95	0.527	F x S	0.24	1.28	0.320	F x S	0.32
Barley	♂	4	2.31	0.745			2.06	0.453		

In the caecal digesta short chain fatty acid concentrations (mMol/L) (Table 61) were altered by changing the diet. Acetic and total SCFA's were reduced ($p < 0.05$) on the wheat diet while acetic acid was reduced ($p < 0.05$) and *n*-valeric acid increased ($p < 0.05$) on the rice diet. The barley diet produced more complex changes with males producing more ($p < 0.05$) acetic, *n*-butyric and total acids then females, males produced some ($p < 0.05$) hexanoic acid while females produced none, *n*-valeric acid was greater ($p < 0.05$) in both sexes fed the barley diet and males on the commercial diet than females on the commercial diet. Log transformed data indicated that *iso*-valeric acid concentration was reduced ($p < 0.05$) when the barley diet was fed to the birds.

Fatty acid proportions of total SCFA in the caecal digesta (Table 62) were affected by each diet. On the wheat diet *n*-valeric acid was increased ($p < 0.05$) compared with the commercial diet. The rice diet increased ($p < 0.05$) *iso*-butyric and *n*-valeric acids while *iso*-valeric acid was lower ($p < 0.05$) in females on the commercial diet compared with males or both sexes on the rice diet. The barley diet increased ($p < 0.05$) *n*-valeric acid, males had higher ($p < 0.05$) *n*-butyric acid and females higher ($p < 0.05$) *iso*-valeric acid in caecal digesta.

Table 61. **Influence of feeding an unsterile commercial grower then irradiated ground wheat, rice or barley diets to SPF leghorns on the concentration (mMol/L) of short chain fatty acids (C2-C7) in caecal digesta at 59 and 62 d old.**

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	**151.152**	5.297	38.629	0.389	2.626	0.478	0.027	0	**198.598**
Commercial	♂	6	**193.330**	6.153	49.233	0.512	2.655	0.631	0	0	**252.514**
Wheat	♀	12	**114.809**	5.557	35.909	0.394	2.992	0.501	0	0	**160.162**
Wheat	♂	7	**120.034**	5.081	38.127	0.529	3.060	0.325	0	0	**167.156**
SE			14.1221	1.0720	6.6644	0.1104	0.4491	0.1055	0.0079	—	20.4113
			15.2536	1.1579	7.1984	0.1192	0.4851	0.1140	—	—	22.0467
			10.7859	0.8187	5.0900	0.0843	0.3430	0.0806	—	—	15.5894
			14.1221	1.0720	6.6644	0.1104	0.4491	0.1140	—	—	20.4113
P			**0.001**	0.92	0.51	0.68	0.85	0.29	0.052*	—	**0.012**
Commercial	♀	8	**133.540**	9.216	31.626	0.572	**1.586**	0.614	0	0	177.153
Commercial	♂	3	**168.010**	4.036	29.192	0.267	**0.856**	0.100	0	0	202.462
Rice	♀	5	**101.405**	8.116	24.292	0.603	**3.085**	0.452	0	0	137.953
Rice	♂	10	**101.291**	10.081	25.644	0.743	**4.105**	0.771	0	0	142.634
SE			10.1895	1.8895	5.6153	0.1137	0.4993	0.1352	—	—	14.3490
			16.6394	3.0856	9.1698	0.1857	0.8153	0.2207	—	—	23.4319
			12.8888	2.3901	7.1029	0.1438	0.6316	0.1710	—	—	18.1503
			9.1138	1.6900	5.0225	0.1017	0.4466	0.1209	—	—	12.8342
P			**0.006**	0.40	0.82	0.19	**0.002**	0.077*	—	—	0.073*
Commercial	♀	5	**105.183**	4.528	**29.900**	0.510	**2.053**	0.731	**0**	0	**142.905**
Commercial	♂	3	**177.254**	7.979	**53.085**	0.486	**4.241**	0.606	**0.018**	0	**243.668**
Barley	♀	8	**122.542**	8.168	**30.610**	0.504	**3.567**	0.522	**0**	0	**165.913**
Barley	♂	7	**140.915**	5.241	**47.478**	0.201	**3.873**	0.258	**0.045**	0.016	**197.996**
SE			13.4328	1.5203	3.8207	0.1125	0.4257	0.1288	—	—	16.9899
			17.3416	1.9627	4.9325	0.1453	0.5496	0.1663	0.0156	—	21.9339
			10.6195	1.2019	3.0205	0.0890	0.3366	0.1019	—	—	13.4317
			11.3528	1.2849	3.2291	0.0951	0.3598	0.1089	0.0102	0.0089	14.3591
P			**0.022**	0.20	**0.001**	0.11	**0.012**	0.064*	**0.018**	0.54	**0.008**

Table 62. Influence of feeding an unsterile commercial grower then irradiated ground wheat, rice or barley diets to SPF leghorns on the short chain fatty acid proportion (%) of total short chain fatty acid (C2-C7) in caecal digesta at 59 and 62 d old.

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	0.781	0.024	0.177	0.002	**0.012**	0.003	tr	0	n/a
Commercial	♂	6	0.779	0.025	0.182	0.002	**0.010**	0.003	0	0	
Wheat	♀	12	0.716	0.036	0.223	0.003	**0.019**	0.003	0	0	
Wheat	♂	7	0.715	0.033	0.229	0.003	**0.018**	0.002	0	0	
SE			0.0253	0.0055	0.0230	0.0006	0.0015	0.0007	—	—	
			0.0273	0.0060	0.0248	0.0006	0.0017	0.0007	—	—	
			0.0193	0.0043	0.0175	0.0004	0.0012	0.0005	—	—	
			0.0253	0.0055	0.0230	0.0006	0.0015	0.0007	—	—	
P			0.09	0.30	0.24	0.56	**0.001**	0.42	—	—	
Commercial	♀	8	0.766	0.054	0.165	**0.003**	**0.008**	**0.004**	0	0	
Commercial	♂	3	0.831	0.020	0.143	**0.001**	**0.004**	**0.001**	0	0	
Rice	♀	5	0.737	0.061	0.172	**0.004**	**0.023**	**0.004**	0	0	
Rice	♂	10	0.708	0.067	0.186	**0.005**	**0.029**	**0.005**	0	0	
SE			0.0245	0.0091	0.0271	0.0006	0.0019	0.0008	—	—	
			0.0339	0.0148	0.0442	0.0010	0.0031	0.0013	—	—	
			0.0309	0.0115	0.0342	0.0008	0.0024	0.0010	—	—	
			0.0219	0.0081	0.0242	0.0005	0.0017	0.0007	—	—	
P			0.065*	0.073*	0.84	**0.015**	**0.001**	**0.031**	—	—	
Commercial	♀	5	0.736	0.032	**0.210**	0.004	**0.014**	**0.005**	0	0	
Commercial	♂	3	0.725	0.033	**0.219**	0.002	**0.018**	**0.003**	tr	0	
Barley	♀	8	0.736	0.051	**0.185**	0.003	**0.021**	**0.003**	0	0	
Barley	♂	7	0.711	0.026	**0.241**	0.001	**0.020**	**0.001**	tr	tr	
SE			0.0157	0.0073	0.0143	0.0008	0.0016	0.000	—	—	
			0.0202	0.0095	0.0184	0.0011	0.0020	0.000	—	—	
			0.0124	0.0058	0.0113	0.0006	0.0012	0.000	—	—	
			0.0132	0.0062	0.0121	0.0007	0.0013	0.000	—	—	
P			0.52	0.083*	**0.023**	0.086*	**0.017**	**0.043**	—	—	

Trial 6.

On the wheat diet, digesta pH (Table 63) in the gizzard and jejunum was reduced ($p < 0.05$) and was lower ($p < 0.05$) in the jejunum of males than in females. When rice was fed, crop and gizzard digesta pH was lower ($p < 0.05$) in females fed the rice diet than males fed the rice or birds of both sexes on the commercial diet. Ileal digesta pH was lower ($p < 0.05$) in birds fed rice and in males. The barley diet reduced ($p < 0.05$) crop, gizzard, jejunal and ileal digesta pH and males had lower ($p < 0.05$) ileal digesta pH.

Table 63. Influence of unsterile commercial grower or irradiated whole wheat, rice or barley diets on the digesta pH and body weight of SPF leghorns at 69 and 73 d old.

Feed	Sex	n	Crop	Gizzard	Jejunum	Ileum	Caeca	Body wt.
Commercial	♀	10	4.86	**4.09** ^{a}	**6.58** ^{ax}	8.36	6.02	**729** ^{by}
Commercial	♂	8	4.99	**4.01** ^{a}	**6.48** ^{ay}	8.13	5.86	**903** ^{bx}
Wheat	♀	8	4.68	**2.86** ^{b}	**6.47** ^{bx}	8.33	5.85	**803** ^{ay}
Wheat	♂	8	4.52	**2.74** ^{b}	**6.25** ^{by}	8.10	6.22	**1007** ^{ax}
SE			0.132	0.125	0.067	0.084	0.214	28.3
			0.148	0.140	0.075	0.094	0.239	31.7
			0.187	0.140	0.075	0.100	0.239	31.7
			0.148	0.150	0.075	0.094	0.239	31.7
P			0.15	**0.01**	**0.02**	0.12	0.67	**0.01**
Commercial	♀	6	**5.77** ^{a}	**4.09** ^{a}	6.54	**8.72** ^{ax}	6.46	**785** ^{b}
Commercial	♂	10	**5.55** ^{a}	**4.07** ^{a}	6.33	**8.28** ^{ay}	6.84	**982** ^{a}
Rice	♀	6	**4.83** ^{b}	**3.08** ^{b}	6.30	**8.37** ^{bx}	6.93	**730** ^{b}
Rice	♂	9	**5.47** ^{a}	**3.83** ^{a}	6.18	**8.05** ^{by}	6.63	**1027** ^{a}
SE			0.220	0.197	0.115	0.156	0.183	26.6
			0.171	0.153	0.089	0.121	0.142	20.6
			0.220	0.197	0.115	0.156	0.183	26.6
			0.180	0.161	0.094	0.127	0.149	21.7
P			**0.03**	**0.02**	0.13	**0.02**	0.24	**0.01**
Commercial	♀	8	**6.34** ^{a}	**4.63** ^{a}	**6.52** ^{a}	**9.01** ^{ax}	6.92	**817** ^{y}
Commercial	♂	7	**6.26** ^{a}	**4.55** ^{a}	**6.45** ^{a}	**8.43** ^{ay}	6.71	**991** ^{x}
Barley	♀	7	**4.98** ^{b}	**2.80** ^{b}	**6.23** ^{b}	**8.19** ^{bx}	6.67	**805** ^{y}
Barley	♂	9	**5.18** ^{b}	**2.96** ^{b}	**6.09** ^{b}	**8.08** ^{by}	6.89	**1078** ^{x}
SE			0.160	0.197	0.099	0.156	0.157	26.6
			0.171	0.153	0.106	0.121	0.168	20.6
			0.171	0.197	0.106	0.156	0.168	26.6
			0.151	0.161	0.094	0.127	0.148	21.7
P			**0.01**	**0.01**	**0.02**	**0.01**	0.61	**0.01**

Superscripts refer to main effects of diet (a, b) or sex (x, y).

Caecal digesta L- or D-lactic acid (Table 64) were not influenced ($p > 0.05$) by feed or sex on any of the wheat, rice or barley feeds. However, a feed x sex interaction approached significance on the rice diet. The commercial diet maintained, and females produced, lower ($p < 0.05$) L-lactic acid concentrations in the ileal digesta than did the wheat diet and males. Significant differences in L- and D-lactic acid were not found across diet or sex when either rice or barley diets were substituted for the commercial diet.

Table 64. Influence of feeding an unsterile commercial grower then irradiated whole wheat, rice or barley diets to SPF leghorns on the concentration (mMol/L) of L- and D-lactic acid in caecal digesta at 69 and 73 d old.

Feed	Sex	n	Ileum		Caeca	
			L-lactic acid	D-lactic acid	L-lactic acid	D-lactic acid
Commercial	♀	7	**8.92** ^{by}	1.29	3.45	3.78
Commercial	♂	8	**16.43** ^{bx}	2.39	2.70	2.70
Wheat	♀	8	**15.38** ^{ay}	3.92	1.20	1.42
Wheat	♂	8	**18.63** ^{ax}	7.06	1.09	1.26
SE			2.148	1.738	1.374	1.129
			2.010	1.626	1.190	0.978
			2.010	1.626	1.272	1.045
			2.010	1.626	1.272	1.045
P			**0.02**	0.10	0.51	0.33
Commercial	♀	6	14.41	0.85	1.16	1.66
Commercial	♂	10	13.38	0.90	0.45	0.77
Rice	♀	6	13.93	5.18	2.40	2.33
Rice	♂	9	21.42	7.66	0.99	1.34
SE			5.570	4.396	1.040	0.906
			3.523	2.780	0.900	0.785
			4.548	3.589	0.735	0.641
			3.713	2.931	0.637	0.555
P			0.41	0.35	0.37	0.47
Commercial	♀	8	6.26	0.27	2.55	2.46
Commercial	♂	7	9.79	0.16	2.24	2.40
Barley	♀	7	16.39	5.43	1.15	1.46
Barley	♂	9	16.14	4.15	1.04	1.23
SE			4.043	2.610	0.437	0.421
			4.668	3.014	0.691	0.465
			4.322	2.791	0.437	0.421
			3.812	2.461	0.369	0.355
P			0.24	0.43	0.06*	0.14

Superscripts refer to main effects of diet (a, b) or sex (x, y).

Ileal digesta short chain fatty acid concentrations (mMol/L) (Table 65) were altered by changing the diet. Propionic acid was reduced ($p < 0.05$) on the wheat diet. Interactions between diet and sex occurred for *iso*-butyric, *n*-valeric, *iso*-valeric and hexanoic acids. On the commercial diet, females produced more ($p < 0.05$) *iso*-butyric than males and birds of either sex produced none on the wheat diet. Females produced more ($p < 0.05$) *n*-valeric on the commercial diet than did males on both diets while females on the wheat diet produced none. Females on the commercial diet produced more ($p < 0.05$) *iso*-valeric acid than males on the wheat diet and males on the commercial, and females on the wheat, diets produced none. Hexanoic acid was only produced ($p < 0.05$) in females on the commercial feed; none was detected in males on the commercial diet or birds of either sex on the wheat diet. The rice diet prevented ($p < 0.05$) propionic acid production, females on the rice produced greater ($p < 0.05$) concentrations of *iso*-butyric acid than females on the commercial diet while males on either diet produced similar and lower ($p < 0.05$) concentrations. Males on the rice diet produced less ($p < 0.05$) *n*-valeric acid than females or birds of either sex on the commercial diet. Total SCFA concentration was greater ($p < 0.05$) in females fed the rice diet than males or birds of either sex on the commercial diet. Males on the barley diet produced greater ($p < 0.05$) acetic, propionic, *n*- and *iso*-butyric, *n*- and *iso*-valeric and total acids than either females on the barley diet or birds of either sex on the commercial diet all of which produced similar ($p > 0.05$) amounts of each acid.

Proportions of total SCFA in the ileal digesta (Table 66) were influenced by the diet changes. Wheat reduced ($p < 0.05$) propionic and *iso*-butyric acids. Both sexes on the commercial and males on the wheat diets produced more ($p < 0.05$) *n*-butyric acid than females on the wheat diet. Females on the commercial

diet produced more ($p < 0.05$) *n*-valeric acid than males on either diet which had greater ($p < 0.05$) concentrations than females on the wheat diet. Females on the commercial diet had higher ($p < 0.05$) concentrations of *iso*-valeric acid than males or either sex on the wheat diet. Hexanoic acid was only found ($p < 0.05$) in females on the commercial diet. Propionic acid was greater ($p < 0.05$) in females on the commercial diet and males on the rice diet than males on the commercial diet or females on the rice diet. Males on the commercial diet had more ($p < 0.05$) *iso*-butyric acid than females on the commercial diet or males on the rice which, in turn, had more ($p < 0.05$) than females on the rice diet which had no measurable concentration. Males on the rice diet had a similar ($p > 0.05$) concentration of *n*-valeric acid to females on the commercial diet though greater ($p < 0.05$) than males on the commercial diet. Both sexes on the commercial diet had similar ($p > 0.05$) concentrations of *n*-valeric acid. Females on the rice diet produced no ($p < 0.05$) *n*-valeric acid. Males on the rice diet produced more *iso*-valeric acid than males on the commercial diet and the females on either diet which produced no measurable concentration. Propionic acid was only found in birds on the commercial diet and not the barley diet. Females produced similar ($p > 0.05$) amounts of *iso*-butyric acid but this was greater ($p < 0.05$) in those on the barley diet than males on either diet. Males on the barley diet had lower ($p < 0.05$) concentrations of *n*-valeric acid than females or birds of either sex on the commercial diet.

Caecal digesta short chain fatty acid concentrations (mMol/L) (Table 67) were altered by changing the diet. The wheat diet produced less ($p < 0.05$) acetic acid than did the commercial diet. Females on the commercial diet had the greatest ($p < 0.05$) concentration of *iso*-butyric acid than other birds, males on the commercial and wheat diet had similar ($p > 0.05$) concentrations while females on the wheat diet had lower ($p < 0.05$) concentrations than all but males on the wheat diet. The wheat diet produced more ($p < 0.05$) *n*-valeric acid than the commercial diet and males on the wheat diet produced more ($p < 0.05$) *iso*-valeric acid than females and birds on the commercial diet. Females on the commercial diet produced the only measurable concentrations ($p < 0.05$) of hexanoic acid. Males on the commercial diet produced similar quantities ($p > 0.05$) of acetic acid to females but greater ($p < 0.05$) than birds on the rice diet. Females on either diet produced similar ($p > 0.05$) amounts of acetic acid but the males on the rice diet produced lower ($p < 0.05$) concentrations than the females on the commercial diet. Males on the commercial diet had greater ($p < 0.05$) concentrations of propionic acid to males on the rice diet but similar ($p > 0.05$) amounts to the females on either diet which, in turn, had similar ($p > 0.05$) amounts to the males on the rice diet. Males on the commercial diet produced more ($p < 0.05$) *n*-butyric and total acids than other birds. The rice diet produced more ($p < 0.05$) of *iso*-butyric acid than the commercial diet. Males on the rice diet produced more ($p < 0.05$) *iso*-valeric acid than other birds. Responses to barley were simpler with a reduction ($p < 0.05$) of acetic and total acids and an increase ($p < 0.05$) in propionic and *n*- and *iso*-valeric acids in birds changed to the barley diet.

Fatty acid proportions in the caecal digesta (Table 68) were affected by each diet. The proportion of acetic acid was reduced ($p < 0.05$) but *n*-valeric acid increased ($p < 0.05$) when the wheat diet was fed. Females on the commercial diet had a greater ($p < 0.05$) proportion of *iso*-butyric acid in caecal digesta than males or birds of either sex on the wheat diet. The rice diet increased ($p < 0.05$) propionic, *n*-valeric and *iso*-valeric acids, males produced more ($p < 0.05$) *n*-butyric acid than females, males on the rice diet produced more ($p < 0.05$) *iso*-butyric acid than females or birds of either sex on the commercial diet. The proportion of acetic acid was greater ($p < 0.05$) in females on the commercial diet than males or birds of either sex on the rice diet. The barley diet produced less ($p < 0.05$) acetic acid but more ($p < 0.05$) propionic, *iso*- or n-valeric acid than the commercial diet.

Table 65. Influence of feeding an unsterile commercial grower then irradiated whole wheat, rice or barley diets to SPF leghorns on the concentration (mMol/L) of short chain fatty acids (C2-C7) in ileal digesta at 69 and 73 d old.

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	9.707	**0.658**	1.018	**1.411**	**0.381**	**0.368**	**0.310**	0.238	14.091
Commercial	♂	8	7.439	**0.368**	0.133	**0.837**	**0.059**	**0**	**0**	0	8.836
Wheat	♀	8	4.702	**0**	0	**0**	**0**	**0**	**0**	0	4.702
Wheat	♂	8	6.076	**0.099**	1.180	**0**	**0.107**	**0.013**	**0**	0	7.475
SE			1.9667	0.1527	0.4632	0.1102	0.0975	0.0752	0.0822	0.0769	2.3804
			1.8397	—	0.4333	0.1031	0.0912	—	—	—	2.2267
			1.8397	0.1428	—	—	—	—	—	—	2.2267
			1.8397	0.1428	0.4333	—	0.0912	0.0703	—	—	2.2267
P			0.31	**0.02**	0.16	**0.001**	**0.043**	**0.003**	**0.025**	0.08	0.054*
Commercial	♀	8	5.061	**0.348**	0.134	**0.639**	**0.173**	0.019	0	0	**6.374**
Commercial	♂	7	5.835	**0.401**	0.288	**0.364**	**0.144**	0.048	0	0	**7.080**
Rice	♀	7	9.120	**0**	0.097	**1.286**	**0.210**	0.037	0	0	**10.749**
Rice	♂	9	5.982	**0**	0.070	**0.304**	**0.018**	0.029	0	0	**6.402**
SE			1.0645	0.0503	0.0609	0.0724	0.0253	0.0229	—	—	1.1116
			1.1380	0.0538	0.0652	0.0774	0.0271	0.0244	—	—	1.1883
			1.1380	—	0.0652	0.0774	0.0271	0.0244	—	—	1.1883
			1.0036	—	0.0575	0.0683	0.0239	0.0215	—	—	1.0480
P			0.07	**0.001**	0.10	**0.001**	**0.001**	0.84	—	—	**0.038**
Commercial	♀	6	**8.156**	**0.276**	**0**	**0.283**	**0.066**	**0**	0	0	**8.781**
Commercial	♂	10	**3.742**	**0.057**	**0.052**	**0.427**	**0.009**	**0.010**	0	0	**4.297**
Barley	♀	6	**3.102**	**0**	**0**	**0**	**0**	**0**	0	0	**3.102**
Barley	♂	9	**17.243**	**0.895**	**1.092**	**1.192**	**0.278**	**0.229**	0	0.001	**20.931**
SE			1.9272	0.1492	—	0.1657	0.0230	—	—	—	2.0917
			1.4928	0.1156	0.1991	0.1284	0.0178	0.0177	—	—	1.6203
			1.9272	—	—	—	—	—	—	—	2.0917
			1.5736	0.1218	0.2099	0.1353	0.0188	0.0186	—	0.0007	1.7079
P			**0.001**	**0.001**	**0.003**	**0.001**	**0.001**	**0.001**	—	0.51	**0.001**

Table 66. Influence of feeding an unsterile commercial grower then irradiated whole wheat, rice or barley diets to SPF leghorns on the short chain fatty acid proportion (%) of total short chain fatty acid (C2-C7) in ileal digesta at 69 and 73 d old.

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	0.687	**0.038**	**0.056**	**0.136**	**0.027**	**0.027**	**0.021**	0.013	n/a
Commercial	♂	8	0.727	**0.040**	**0.138**	**0.089**	**0.006**	**0**	**0**	0	
Wheat	♀	8	0.750	**0**	**0**	**0**	**0**	**0**	**0**	0	
Wheat	♂	8	0.740	**0.006**	**0.120**	**0**	**0.008**	**0.001**	**0**	0	
SE			0.0865	0.0085	0.0452	0.0198	0.0045	0.0035	**0.0025**	0.0024	
			0.0809	0.0079	0.0423	0.0185	0.0042	—	—	—	
			0.0809	—	—	—	—	—	—	—	
			0.0809	0.0079	0.0423	—	0.0042	0.0033	—	—	
P			0.81	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	0.10	
Commercial	♀	8	0.912	**0.027**	0	**0.053**	**0.008**	**0**	0	0	
Commercial	♂	7	0.860	**0.006**	0.011	**0.121**	**0.002**	**0.001**	0	0	
Rice	♀	7	0.833	**0**	0	**0**	**0**	**0**	0	0	
Rice	♂	9	0.798	**0.046**	0.052	**0.077**	**0.017**	**0.010**	0	0	
SE			0.0934	0.0092	—	0.0214	0.0048	—	—	—	
			0.0724	0.0071	0.0378	0.0166	0.0038	0.0029	—	—	
			0.0634	—	—	—	—	—	—	—	
			0.0763	0.0075	0.0399	0.0174	0.0040	0.0031	—	—	
P			0.81	**0.01**	0.31	**0.01**	**0.01**	**0.01**	—	—	
Commercial	♀	6	0.795	**0.051**	0.018	**0.107**	**0.028**	0.003	0	0	
Commercial	♂	10	0.830	**0.053**	0.038	**0.055**	**0.019**	0.005	0	0	
Barley	♀	6	0.837	**0**	0.010	**0.128**	**0.021**	0.004	0	0	
Barley	♂	9	0.928	**0**	0.013	**0.048**	**0.002**	0.008	0	tr	
SE			0.0809	0.0073	0.0143	0.0185	0.0042	0.0033	—	—	
			0.0865	0.0095	0.0184	0.0198	0.0045	0.0035	—	—	
			0.0865	—	0.0113	0.0198	0.0045	0.0035	—	—	
			0.0763	—	0.0121	0.0174	0.0040	0.0031	—	—	
P			0.81	**0.01**	0.31	**0.01**	**0.01**	0.23	—	—	

Table 67. Influence of feeding an unsterile commercial grower then irradiated whole wheat, rice or barley diets to SPF leghorns on the concentration (mMol/L) of short chain fatty acids (C2-C7) in caecal digesta at 69 and 73 d old.

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	**96.403**	9.887	21.691	**0.993**	**1.727**	**0.448**	**0.290**	0.182	131.620
Commercial	♂	8	**114.269**	14.812	29.964	**0.685**	**2.271**	**0.382**	**0.025**	0	162.407
Wheat	♀	8	**91.326**	14.039	35.087	**0.380**	**3.499**	**0.406**	**0.015**	0	144.752
Wheat	♂	8	**84.654**	14.758	31.271	**0.631**	**4.142**	**0.777**	**0.042**	0	136.274
SE			7.8901	1.6349	4.8818	0.1018	0.4139	0.1059	0.0741	0.0616	11.2129
			7.3805	1.5293	4.5666	0.0953	0.3871	0.0990	0.0693	—	10.4887
			7.3805	1.5293	4.5666	0.0953	0.3871	0.0990	0.0693	—	10.4887
			7.3805	1.5293	4.5666	0.0953	0.3871	0.0990	0.0693	—	10.4887
P			**0.050**	0.12	0.26	**0.002**	**0.001**	**0.030**	**0.037**	0.11	0.21
Commercial	♀	8	**119.956**	**16.646**	**29.251**	**0.316**	3.016	**0.279**	0.020	0	**169.484**
Commercial	♂	7	**146.984**	**20.716**	**55.724**	**0.375**	4.300	**0.362**	0.039	0	**228.501**
Rice	♀	7	**108.660**	**19.216**	**35.729**	**0.635**	4.492	**0.777**	0.031	0	**169.540**
Rice	♂	9	**84.541**	**15.642**	**33.356**	**1.099**	4.045	**1.285**	0.061	0	**140.030**
SE			11.9377	1.4747	5.1496	0.1488	0.4144	0.1508	0.0127	—	16.9873
			11.0522	1.3653	4.7676	0.1378	0.3837	0.1396	0.0117	—	15.7272
			11.0522	1.3653	4.7676	0.1378	0.3837	0.1396	0.0117	—	15.7272
			9.7471	1.2041	4.2046	0.1215	0.3384	0.1231	0.0103	—	13.8701
P			**0.003**	**0.043**	**0.003**	**0.001**	0.07	**0.001**	0.09	—	**0.003**
Commercial	♀	6	**125.998**	**13.974**	40.945	0.862	**3.251**	**0.462**	0.011	0	**185.493**
Commercial	♂	10	**151.149**	**11.841**	37.122	0.745	**1.978**	**0.736**	0.041	0	**203.612**
Barley	♀	6	**91.023**	**19.848**	25.439	0.699	**4.337**	**0.842**	0.009	0	**142.196**
Barley	♂	9	**88.621**	**21.112**	25.959	0.998	**4.869**	**1.336**	0.018	0	**142.911**
SE			14.9185	2.2167	6.0635	0.1730	0.5772	0.1978	0.0132	—	17.7841
			12.1809	1.8099	4.9508	0.1412	0.1413	0.1615	0.0110	—	14.5206
			14.9185	2.2167	6.0635	0.1730	0.5772	0.1978	0.0132	—	17.7841
			14.9185	2.2167	6.0635	0.1730	0.5772	0.1978	0.0132	—	17.7841
P			**0.009**	**0.010**	0.18	0.60	**0.003**	**0.033**	0.21	—	**0.029**

Table 68. Influence of feeding an unsterile commercial grower then irradiated whole wheat, rice or barley diets to SPF leghorns on the short chain fatty acid proportion (%) of total short chain fatty acid (C2-C7) in caecal digesta at 69 and 73 d old.

Diet	Sex	n	Short chain fatty acid (C2-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Commercial	♀	7	**0.732**	0.078	0.160	**0.008**	**0.014**	0.004	0.003	0.002	n/a
Commercial	♂	8	**0.714**	0.093	0.173	**0.004**	**0.013**	0.002	0	0	
Wheat	♀	8	**0.630**	0.097	0.243	**0.003**	**0.024**	0.003	0	0	
Wheat	♂	8	**0.618**	0.109	0.233	**0.005**	**0.031**	0.006	<0.001	0	
SE			0.0312	0.0107	0.0262	0.0010	0.0025	0.0010	0.0008	0.0007	
			0.0292	0.0101	0.0245	0.0010	0.0024	0.0010	—	—	
			0.0292	0.0101	0.0245	0.0010	0.0024	0.0010	—	—	
			0.0292	0.0101	0.0245	0.0010	0.0024	0.0010	0.0008	—	
P			**0.02**	0.24	0.06*	**0.01**	**0.01**	0.06*	0.07*	0.19	
Commercial	♀	8	**0.703**	**0.099**	**0.177**	**0.002**	**0.018**	**0.002**	0	0	
Commercial	♂	7	**0.644**	**0.092**	**0.242**	**0.002**	**0.019**	**0.002**	0	0	
Rice	♀	7	**0.637**	**0.117**	**0.209**	**0.004**	**0.027**	**0.005**	<0.001	0	
Rice	♂	9	**0.604**	**0.112**	**0.237**	**0.008**	**0.029**	**0.010**	<0.001	0	
SE			0.0178	0.0055	0.0174	0.00164	0.0021	0.0015	—	—	
			0.0164	0.0051	0.0161	0.0011	0.0019	0.0013	—	—	
			0.0164	0.0051	0.0161	0.0011	0.0019	0.0013	0.0001	—	
			0.0145	0.0045	0.0142	0.0009	0.0017	0.0012	0.0001	—	
P			**0.01**	**0.01**	**0.04**	**0.01**	**0.01**	**0.01**	0.19	—	
Commercial	♀	6	**0.678**	**0.076**	0.221	0.005	**0.018**	**0.003**	0	0	
Commercial	♂	10	**0.732**	**0.060**	0.190	0.004	**0.011**	**0.004**	<0.001	0	
Barley	♀	6	**0.637**	**0.141**	0.178	0.006	**0.031**	**0.007**	0	0	
Barley	♂	9	**0.616**	**0.143**	0.184	0.008	**0.035**	**0.010**	<0.001	0	
SE			0.0296	0.0091	0.0272	0.0013	0.0031	0.0017	—	—	
			0.0242	0.0074	0.0222	0.0011	0.0025	0.0014	0.0001	—	
			0.0296	0.0091	0.0272	0.0013	0.0031	0.0017	—	—	
			0.0296	0.0091	0.0272	0.0013	0.0031	0.0017	0.0001	—	
P			**0.03**	**0.01**	0.69	0.21	**0.01**	**0.02**	0.64	—	

7.4 Discussion

Unless otherwise specified, commercial SPF flocks are populated with a "normal" gut microbial load; the birds are not, as is often assumed, devoid of gut microbial populations.

A pattern of digesta pH changes changed from short to longer term experiments. In the short-term, ileal pH tended to rise with feed change but this was reversed in the later experiments when longer periods of time between feed change and sampling occurred. In these, ileal digesta pH generally decreased from that in the control birds. Similarly, the concentration of ileal D- and L-lactic acid increased and, unlike in most of the experiments in the current and earlier (Taylor, 2002) work, substantial reductions in SCFA concentration were produced. The changes in SCFA concentration were largely reductions in acetate and, as this contributed most of the total SCFA, total concentrations. However, other changes involved alterations to propionate and butyric acids and the *iso*- forms. Additionally, the proportion of acetic acid of total was reduced substantially across all three cereals, wheat, rice and barley, in the final experiment (experiment 6), confirming the trend suggested by the previous trial (experiment 5). These results are similar to those of Vernia *et al*. (1988a) who, in humans, found that increased lactate concentrations and reduced pH paralleled a reduction in SCFA's. The increased lactic acid concentration may be due to a higher glycolytic flux from increased substrate flow favouring lactate production (Cummings, 1981).

Despite the note made in the introduction of the limited replication across sex leading to artefact in some significant results, consistent trends were noted in altered SCFA production between males and female birds in trials conducted with the range of cereals. This result warrants further investigation as little evidence is presented from any animal or human model that dietary constituents are fermented differently by the sexes. This is despite much conjectural claim that such differences occur and anecdotal evidence, at least from animal work, that fermentation patterns are similar.

Finally, it is recognised by the author that destruction of micro-organism populations on/in the cereals, other raw materials and the finished feeds, produces break-down products that, in themselves, can affect digestive function. However, this occurs in all processed feeds. Steam-conditioned and pelleted feeds might, at least temporarily, be rendered sterile, but soon gain new microbial populations. The intention in these experiments was to use a feed that did not cause the ingestion of a viable microbial population with its potential to supplant and/or interfere with resident gut populations and, thus, affect digestive function. Furthermore, the use of sterilised feeds is part of everyday SPF bird production, with little apparent criticism of the method, and is used often in routine gut microbial studies in chickens and other animals.

8. Effects of cholera vaccine on gut pH, fermentation and tissue in SPF Leghorns.

8.1 Introduction

Fowl cholera is a contagious disease caused by a gram negative bacteria, *Pasteurella multocida* (DPI Qld, 1998). It is found world wide, causes different forms of disease in many animal species and in both domestic and wild birds can cause acute and/or chronic infection (DPI Qld, 1998). In domestic birds hygiene is a major factor in its transmission from flock to flock and while killed vaccines are used, strain differences create many problems with their use (DPI Qld, 1998).

Specific application of autogenous vaccines has provided some good protection within integrated poultry operations but a suitable challenge model for testing of vaccines in Australia eludes those attempting to produce vaccines with wider application and efficacy. Methods of typing have been/are of little practical or scientific use but recent application of the more refined molecular biological techniques is beginning to shed some light on the organism(s). However, to date this has not aided field treatment of the disease which remains complex and frustrating. Live vaccines have been developed by many organisations, even within Australia, but have yet to achieve any useful field application. The CU vaccine in the USA has, despite claims of its general applicability, achieved little general use.

The constituents of many vaccine types may have effects on the gut; both in affecting gut organisms through direct effects, via immune responses, or by indirect effects through alterations to conditions within the gut lumen. Organisms that may constitute part of the vaccine and/or its adjuvants may result in alterations to digestive function.

As part of cholera vaccine application in commercial organisations, a suitable adjuvant is used. The adjuvant of interest to this current nutrition work was derived of a common bacteria found in poultry and was, originally, one of many organisms considered for development. Historically, the company producing the adjuvant became interested in the organism, then known as *Coryne parvum*, noted as a standard adjuvant in the literature. The interest in this adjuvant was that being derived of a standard gut micro-organism, its effect on the gut lumen, through stimulation of an immune response following intra-muscular vaccination, required consideration of its potential to affect microbial populations associated with digestive function. There is little information in the literature on specific cases despite general concern about "disease:nutrition interactions".

The use of specific pathogen-free (SPF) birds and feeds devoid of an active micro-organism population would normally be precluded in nutrition trials because of the expense involved in procuring and maintaining facilities, feeds and birds. The opportunity to use such birds to develop the results obtained in earlier work (Taylor, 2002) arose and enabled development of further work from the acidosis trials to be done with disease free birds and using feed sterility and cholera vaccination.

An organism introduced into the bird for the purposes of creating an immune response that results in protection against a disease, produces a systemic effect which, obviously, includes gut tissue. Therefore, two hypotheses were considered in approaching these trials. Firstly, the use of an adjuvant based on a common gut organism may affect gut function. Secondly, cholera (or any other organism) may, similarly, affect gut function. Alterations in gut function, associated with micro-organism activity, specifically fermentation responses, can be monitored using the markers used in the earlier work.

8.2 Materials and methods

The birds were SPF Leghorn-derived lines (as described above) hatched from SPF eggs and grown from day old, in filtered air positive pressure (FAPP) isolators. Feed and water were provided through aseptic or sterile procedures to the birds. Where sterile, feeds were sterilized with ethylene oxide or gamma irradiation (50 kG kg^{-1} imparted energy). FAPP isolators held unvaccinated control birds and those vaccinated with cholera emulsions of various sub-types. The birds were killed by cervical dislocation after approximately three weeks and inspected for local necrotic reaction in the muscle at the vaccination site.

The cholera vaccines used in the current work were all formalin-inactivated commercial vaccines, of many types. The adjuvant-base organism is grown in isolation, a suspension is made at the required concentration, then incorporated into the vaccine at a specified amount per dose. However, the adjuvant was a standard – only the antigen varied in each vaccine used in each experiment. The vaccines were all made to the same (i.e. a standard) packed-cell volume.

A range of diets were used in the following experiments but limited resources prevented the establishment of factorial experiments to investigate the effects of vaccination and diet simultaneously. Therefore, the following trials only investigate the effect of vaccination within a dietary treatment.

Digesta samples were taken from both groups of birds and treated as described above and by Taylor (2002). Feed intake was not measurable but changes of diets did not cause noticeable alteration to total feed consumption or time for feed to be depleted, reflecting a similar response to diet change noted in all the previous trials.

Experiments

Trial 1: Irradiated commercial starter feed. Birds inoculated (EM 9926 CH#96) at 44 d; killed at 72 d old.

Trial 2: Ethylene oxide sterilised commercial starter feed. Birds in two separate isolators testing different vaccines inoculated (EM 0006 CH#5 and EM 0005 CH#28) at 49 d; killed at 76 d old.

Trial 3: Unsterile commercial starter and grower feed. Birds inoculated (EM 0019 CH#49) at 44 d; killed at 73 d old.

Trial 4: Unsterile commercial starter and grower feed. Birds inoculated (EM 0101 CH#48) at 43 d; killed at 71 d old.

Trial 5: Unsterile commercial starter and grower feed. Birds in two separate isolators testing different vaccines inoculated (EM? CH#54 and EM 0103 CH#55) at 48 d; killed at 76 d old.

Trial 6: Replicated trial with same source birds in a second group i.e. same source flock one month older (Trials 6a and 6b, respectively). Irradiated commercial starter feed. Birds inoculated (EM CHO#4053-001) at 44 d in both replicates; killed at 65 or 71 d old.

8.3 Results

Initial experiments, used to develop consistency, involved 3 trials with cholera vaccinated birds and 2 with HVT vaccinated birds to decide upon appropriate methods for the trials reported here.

Trial 1

Ileal digesta pH (Table 69) was lower ($p < 0.05$) and body weight greater ($p < 0.05$) in males than females. Cholera vaccination did not affect digesta pH nor body weight. Muscle reaction tests were clear.

Table 69. Influence of cholera vaccination (CH#96) on digesta pH of SPF leghorns at 72 d fed continuously on an irradiated commercial starter diet.

Gut section	Vaccination	Sex	n	LS Mean	SE (LS mean)	P
Crop	Control	♀	7	5.19	0.082	0.124
	Control	♂	8	5.19	0.077	
	CH#96	♀	7	5.14	0.082	
	CH#96	♂	8	4.95	0.077	
Gizzard	Control	♀		3.42	0.084	0.072*
	Control	♂		3.56	0.078	
	CH#96	♀		3.71	0.084	
	CH#96	♂		3.70	0.078	
Ileum	Control	♀		7.26^{x}	0.073	**0.004**
	Control	♂		6.89^{y}	0.068	Sex
	CH#96	♀		7.04^{x}	0.073	
	CH#96	♂		6.91^{y}	0.068	
Caeca	Control	♀		5.66	0.077	0.232
	Control	♂		5.48	0.072	
	CH#96	♀		5.46	0.077	
	CH#96	♂		5.48	0.072	
Body weight	Control	♀		708^{y}	25.4	**0.001**
	Control	♂		862^{x}	23.8	Sex
	CH#96	♀		695^{y}	25.4	
	CH#96	♂		861^{x}	23.8	

Trial 2

Gizzard digesta pH (Table 70) was higher ($p < 0.05$) after inoculation with the CH#28 vaccine than in the control birds with birds given the CH#5 vaccine having intermediate pH. Males were heavier ($p < 0.05$) than females. Muscle reaction was clear with the EM 0006 CH#5 vaccine but EM 0005 CH#28 produced a bad reaction.

Table 70. Influence of cholera vaccination (CH#5 and CH#28) on the digesta pH and body weight of SPF leghorns at 76 d old fed continuously on an ethylene oxide sterilized commercial starter diet.

Gut section	Vaccination	Sex	N	Mean	SD (mean)	P
Crop	Control	♀	9	5.63	0.642	0.172
	Control	♂	6	5.82	0.532	
	CH#5	♀	10	5.50	0.323	
	CH#5	♂	4	5.08	0.222	
	Control	♀	9	5.31	0.679	
	Control	♂	6	5.02	0.659	
	CH#28	♀	10	5.24	0.914	
	CH#28	♂	4	5.05	0.191	
Gizzard	Control	♀		3.12^{b}	0.591	**0.010**
	Control	♂		2.90^{b}	0.363	Cholera

	CH#5	♀	3.38 [a]	0.397	
	CH#5	♂	3.25 [a]	0.507	
	Control	♀	3.09 [b]	0.247	
	Control	♂	3.02 [b]	0.299	
	CH#28	♀	3.56 [a]	0.353	
	CH#28	♂	3.55 [a]	0.614	
Ileum	Control	♀	7.82	0.399	0.380
	Control	♂	7.48	0.449	
	CH#5	♀	7.50	0.333	
	CH#5	♂	7.50	0.300	
	Control	♀	7.74	0.635	
	Control	♂	7.83	0.476	
	CH#28	♀	7.55	0.479	
	CH#28	♂	7.33	0.479	
Caeca	Control	♀	6.56	0.181	0.142
	Control	♂	6.28	0.306	
	CH#5	♀	6.60	0.374	
	CH#5	♂	6.48	0.050	
	Control	♀	6.48	0.335	
	Control	♂	6.33	0.294	
	CH#28	♀	6.20	0.323	
	CH#28	♂	6.55	0.208	
Body weight	Control	♀	713 [y]	56.9	**0.001**
	Control	♂	892 [x]	143.8	Sex
	CH#5	♀	726 [y]	50.0	
	CH#5	♂	899 [x]	66.2	
	Control	♀	731 [y]	75.3	
	Control	♂	840 [x]	60.3	
	CH#28	♀	733 [y]	59.5	
	CH#28	♂	901 [x]	53.0	

Trial 3

Ileal digesta pH (Table 71) was reduced ($p < 0.05$) by the cholera vaccine. The vaccine produced a lower ($p < 0.05$) crop pH in females alone as the males had similar ($p > 0.05$) pH to unvaccinated birds. Males were heavier ($p < 0.05$) than females with no effect ($p > 0.05$) of vaccine. Muscle reactions were clear.

Table 71. Influence of cholera vaccination (CH#49) on digesta pH of SPF leghorns at 73 d old fed continuously on unsterilised commercial starter and grower diets.

Gut section	Vaccination	Sex	N	LS Mean	SE (LS mean)	P
Crop	Control	♀	7	6.11 [a]	0.202	**0.006**
	Control	♂	9	5.76 [a]	0.178	V x S
	CH#49	♀	5	4.88 [b]	0.267	

	CH#49	♂	9	6.02 [a]	0.178	
Gizzard	Control	♀		4.06	0.212	0.116
	Control	♂		3.44	0.187	
	CH#49	♀		3.60	0.251	
	CH#49	♂		3.40	0.187	
Ileum	Control	♀		8.49[a]	0.148	**0.001**
	Control	♂		8.47[a]	0.131	Vaccine
	CH#49	♀		7.74[b]	0.175	
	CH#49	♂		7.83[b]	0.131	
Caeca	Control	♀		6.03	0.125	0.146
	Control	♂		5.81	0.111	
	CH#49	♀		6.04	0.148	
	CH#49	♂		5.69	0.111	
Colon	Control	♀		6.55	0.301	0.546
	Control	♂		6.47	0.246	
	CH#49	♀		6.95	0.368	
	CH#49	♂		6.93	0.301	
Body weight	Control	♀		937 [y]	27.2	**0.001**
	Control	♂		1087 [x]	24.0	Sex
	CH#49	♀		915 [y]	32.2	
	CH#49	♂		1151 [x]	24.0	

Trial 4

Ileal digesta pH (Table 72) was reduced ($p < 0.05$) by the cholera vaccine and in males compared with females irrespective of vaccination. Vaccination produced a lower ($p < 0.05$) body weight in both sexes and males were heavier ($p < 0.05$) than females.

Table 72. Influence of cholera vaccination (CH#48) on digesta pH of SPF leghorns at 71 d old fed continuously on unsterilised commercial starter and grower diets.

Gut section	Vaccination	Sex	n	LS Mean	SE (LS mean)	P
Crop	Control	♀		5.61	0.357	0.247
	Control	♂		–	–	
	CH#48	♀		–	–	
	CH#48	♂		5.01	0.252	
Gizzard	Control	♀	8	3.70	0.210	0.634
	Control	♂	7	4.00	0.225	
	CH#48	♀	6	4.03	0.266	
	CH#48	♂	8	3.74	0.210	
Ileum	Control	♀		7.22 [a x]	0.171	**0.012**
	Control	♂		6.63 [a y]	0.183	Vaccine
	CH#48	♀		6.74 [b x]	0.197	Sex
	CH#48	♂		6.35 [b y]	0.171	
Caeca	Control	♀		5.55	0.138	0.187
	Control	♂		5.16	0.148	
	CH#48	♀		5.59	0.160	
	CH#48	♂		5.45	0.138	

Body weight	Control	♀	800 $^{a\,y}$	33.3	**0.001**
	Control	♂	1024 $^{a\,x}$	35.7	Vaccine
	CH#48	♀	730 $^{b\,y}$	38.5	Sex
	CH#48	♂	943 $^{b\,x}$	33.3	

Superscripts refer to main effects of diet (a, b) or sex (x, y).

VFA concentrations in ileal digesta (Table 73) were altered by vaccination with an increase ($p < 0.05$) in *n*-butyric and hexanoic acids. Vaccination did not alter ($p > 0.05$) VFA concentrations in caecal digesta.

In ileal digesta, vaccination reduced ($p < 0.05$) acetic acid and increased ($p < 0.5$) *n*-butyric and hexanoic acid proportions (Table 73). Proportions of VFA's in caecal digesta were unaltered ($p > 0.05$) by vaccination.

Table 73. Influence of cholera vaccination (CH#48) on the concentration (mMol/L) and proportions of short chain fatty acids (C2-C7) in ileal and caecal digesta of SPF leghorns at 71 d old fed continuously on unsterilised commercial starter and grower diets.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Control	♀	7[2]	6.433	0.023	**0**	0	0	0	**0**	0.060	6.516
Concentration	Control	♂	6[3]	5.779	0.050	**0.004**	0	0	0	**<0.001**	0	5.833
	CH#48	♀	6[3]	7.376	0.060	**0.349**	0	0	0	**0.013**	0	7.797
	CH#48	♂	8[1]	8.069	0.077	**0.314**	0.004	0	0	**0.029**	0	8.493
	SE[2]			1.0464	0.0245	—	—	—	—	—	0.0276	1.1422
	SE[3]			1.1303	0.0264	0.1102	—	—	—	0.0065	—	1.2338
	SE[1]			0.9788	0.0229	0.0955	0.0022	—	—	0.0056	—	1.0685
	P			0.45	0.47	**0.037**	0.52	—	—	**0.005**	0.35	0.37
Ileum	Control	♀	7[2]	**0.990**	0.003	**0**	0	0	0	**0**	0.007	N/A
Proportion	Control	♂	6[3]	**0.990**	0.009	**0.001**	0	0	0	**<0.001**	0	
	CH#48	♀	6[3]	**0.957**	0.006	**0.035**	0	0	0	**0.002**	0	
	CH#48	♂	8[1]	**0.955**	0.008	**0.033**	<0.001	0	0	**0.004**	0	
	SE[2]			0.0102	0.0030	—	—	—	—	—	0.0026	
	SE[3]			0.0110	0.0032	0.0087	—	—	—	0.0009	—	
	SE[1]			0.0095	0.0028	0.0076	0.0002	—	—	0.0008	—	
	P			**0.029**	0.59	**0.005**	0.52	—	—	**0.008**	0.22	
Caeca	Control	♀	8[1]	104.091	3.870	13.061	0.089	0.475	0.173	0	0.099	121.858
Concentration	Control	♂	7[2]	116.174	2.535	14.394	0	0.163	0	0	0	133.265
	CH#48	♀	6[3]	115.730	4.345	18.859	0	0.641	0	0	0	139.575
	CH#48	♂	8[1]	125.843	5.147	16.506	0.107	0.356	0.232	0	0	148.192
	SE[1]			8.0829	0.8031	2.8462	0.0737	0.1558	0.0810	—	0.0526	9.2225
	SE[2]			8.6409	0.8586	3.0427	—	0.1666	—	—	—	9.8593
	SE[3]			9.3333	0.9274	3.2865	—	0.1799	—	—	—	10.6493
	P			0.33	0.19	0.57	0.66	0.27	0.15	—	0.47	0.26
Caeca	Control	♀	8[1]	0.757	0.041	0.189	0.001	0.008	0.002	0	0.002	N/A
Proportion	Control	♂	7[2]	0.806	0.021	0.171	0	0.002	0	0	0	
	CH#48	♀	6[3]	0.769	0.038	0.186	0	0.008	0	0	0	
	CH#48	♂	8[1]	0.760	0.040	0.187	0.002	0.009	0.003	0	0	
	SE[1]			0.0140	0.0064	0.0083	0.0011	0.0021	0.0013	—	0.0010	
	SE[2]			0.0149	0.0068	0.0089	—	0.0023	—	—	—	
	SE[3]			0.0161	0.0074	0.0096	—	0.0025	—	—	—	
	P			0.10	0.17	0.46	0.65	0.21	0.25	—	0.47	

Trial 5

Jejunal and ileal digesta pH (Table 74) was lower ($p < 0.05$) after inoculation with either vaccine. The CH#54 vaccine produced a lower ($p < 0.05$) caecal digesta pH than the CH#55 vaccine or in control birds. Ileal pH was lower ($p < 0.05$) in males. Males were heavier ($p < 0.05$) than females and there was no effect ($p > 0.05$) of vaccine.

Table 74. Influence of cholera vaccination (CH#54 and CH#55) on the digesta pH and body weight of SPF leghorns at 76 d old fed continuously on an unsterilised commercial starter and grower diet.

Gut section	Vaccination	Sex	N	Mean	SD (mean)	P
Crop	Control	♀	7	5.18	0.172	0.144
	Control	♂	7	4.79	0.130	
	CH#54	♀	7	4.93	0.172	
	CH#54	♂	8	4.69	0.121	
	Control	♀	7	5.63	0.343	
	Control	♂	8	4.91	0.121	
	CH#55	♀	7	4.78	0.130	
	CH#55	♂	8	4.80	0.140	
Jejunum	Control	♀		6.34 [a]	0.146	**0.003**
	Control	♂		6.47 [a]	0.146	Vaccine
	CH#54	♀		6.21 [b]	0.157	
	CH#54	♂		6.21 [b]	0.136	
	Control	♀		7.12 [a]	0.146	
	Control	♂		6.52 [a]	0.136	
	CH#55	♀		6.14 [b]	0.146	
	CH#55	♂		6.20 [b]	0.136	
Ileum	Control	♀		8.22 [ax]	0.112	**0.001**
	Control	♂		7.62 [ay]	0.112	Vaccine
	CH#54	♀		7.58 [bx]	0.112	Sex
	CH#54	♂		7.21 [by]	0.105	
	Control	♀		8.29 [ax]	0.112	
	Control	♂		7.90 [ay]	0.105	
	CH#55	♀		7.59 [bx]	0.112	
	CH#55	♂		7.34 [by]	0.105	
Caeca	Control	♀		5.69 [a]	0.190	**0.008**
	Control	♂		5.78 [a]	0.190	Vaccine
	CH#54	♀		5.39 [b]	0.190	
	CH#54	♂		5.56 [b]	0.177	
	Control	♀		6.16 [a]	0.190	
	Control	♂		6.38 [a]	0.177	
	CH#55	♀		5.85 [a]	0.190	
	CH#55	♂		6.02 [a]	0.177	
Body weight	Control	♀		931 [y]	34.0	**0.001**
	Control	♂		1197 [x]	34.0	Sex
	CH#54	♀		882 [y]	34.0	
	CH#54	♂		1123 [x]	31.8	
	Control	♀		905 [y]	34.0	
	Control	♂		1173 [x]	31.8	
	CH#55	♀		910 [y]	34.0	
	CH#55	♂		1129 [x]	31.8	

VFA concentrations in ileal digesta (Table 75) varied across the two cholera vaccines. Acetic acid and total VFA's increased ($p < 0.05$) with CH#54 vaccination and vaccinated males produced more ($p < 0.05$) propionic and *iso*-butyric acids than females or both sexes of the control birds. Cholera #55 vaccine produced less ($p < 0.05$) *iso*-butyric acid in ileal digesta than did control birds. In caecal digesta, cholera #54 produced less ($p < 0.05$) *iso*-butyric acid but more ($p < 0.05$) *n*-butyric and total VFA's than in unvaccinated birds. Males produced more ($p < 0.05$) acetic, *n*-butyric and total acids than females. Cholera #55 increased ($p < 0.05$) acetic and total acids than in control birds. Unvaccinated females produced more ($p < 0.05$) hexanoic and heptanoic acids than did males or vaccinated birds. Males produced more ($p < 0.05$) acetic, propionic and total acids than females.

Cholera#54 produced a lesser ($p < 0.05$) proportion (Table 76) of acetic acid in ileal digesta while propionic and *iso*-butyric acids were greater ($p < 0.05$) in vaccinated males compared with females or unvaccinated birds. Proportions of VFA's were unaltered ($p > 0.05$) by CH#55 vaccine. Caecal digesta contained a greater ($p < 0.05$) proportion of *n*-butyric acid in birds vaccinated with CH#54 while unvaccinated females had a greater ($p < 0.05$) proportion of *iso*-butyric acid than males or vaccinated birds. Unvaccinated females had greater ($P < 0.05$) proportions of both hexanoic and heptanoic acids than did males or birds vaccinated with CH#55.

Table 75. Influence of cholera vaccination (CH#54 and CH#55) on the concentration (mMol/L) of short chain fatty acids (C2-C7) in ileal and caecal digesta of SPF leghorns at 76 d old fed continuously on unsterilised commercial starter and grower diets.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Control	♀	7[2]	**3.491**	**0**	0	**0**	0	0	0	0	**3.495**
	Control	♂	7[2]	**5.680**	**0**	0	**0**	0	0	0	0	**5.684**
	CH#54	♀	7[2]	**7.099**	**0.018**	0.331	**0**	0	0	0	0	**7.451**
	CH#54	♂	8[1]	**9.684**	**0.198**	0.531	**0.079**	0.051	0.028	0.030	0.059	**10.668**
	SE[2]			1.4954	0.0371	0.1773	—	—	—	—	—	1.6019
	SE[1]			1.4954	0.0371	0.1773	0.0172	0.0175	0.0142	0.0151	0.0155	1.6019
	P			**0.050**	**0.002**	0.12	**0.006**	0.12	0.41	0.41	0.07	**0.029**
Ileum	Control	♀	7[2]	9.551	0.060	0.117	**0.051**	0	0.006	0.027	0.013	9.830
	Control	♂	8[1]	7.563	0.054	0.008	**0.006**	0	0	0	0	7.635
	CH#55	♀	7[2]	7.257	0.036	0	**0**	0	0	0	0	7.297
	CH#55	♂	8[1]	8.698	0.002	0.094	**0**	0	0	0	0	8.798
	SE[2]			1.3074	0.0321	0.0722	0.0134	—	0.0027	0.0081	0.0039	1.3314
	SE[1]			1.2230	0.0300	0.0676	0.0125	—	—	—	—	1.2454
	P			0.58	0.54	0.55	**0.029**	—	0.36	0.06	0.07	0.52
Caeca	Control	♀	7[2]	**65.617**	2.681	**15.627**	**0.636**	0.818	0.227	0.013	0	**85.623**
	Control	♂	7[2]	**90.766**	4.162	**27.200**	**0.553**	1.451	0.264	0.023	0.001	**124.425**
	CH#54	♀	7[2]	**81.914**	3.495	**26.179**	**0.511**	1.118	0.194	0	0.001	**113.416**
	CH#54	♂	8[1]	**98.045**	3.240	**31.179**	**0.503**	1.010	0.179	0.014	0	**134.174**
	SE[2]			6.0630	0.4669	2.3442	0.0323	0.1942	0.0412	0.0126	0.0010	8.3292
	SE[1]			5.6714	0.4368	2.1928	0.0302	0.1816	0.0386	0.0118	—	7.7913
	P			**0.005**	0.19	**0.001**	**0.026**	0.16	0.47	0.65	0.55	**0.002**
Caeca	Control	♀	7[2]	**57.991**	**2.150**	12.596	0.648	0.919	0.518	**0.397**	**0.402**	**75.624**
	Control	♂	8[1]	**81.485**	**3.994**	16.993	0.454	1.035	0.370	**0.068**	**0.029**	**104.435**
	CH#55	♀	7[2]	**70.819**	**2.966**	23.169	0.936	1.598	0.791	**0.110**	**0.006**	**100.397**
	CH#55	♂	8[1]	**96.011**	**4.900**	21.761	0.694	1.263	0.559	**0.067**	**0**	**125.261**
	SE[2]			4.3634	0.4542	3.8119	0.1241	0.2975	0.1867	0.0716	0.0673	6.3475
	SE[1]			4.0815	0.4249	3.5657	0.1161	0.2783	0.1747	0.0670	0.0629	5.9375
	P			**0.001**	**0.001**	0.21	0.07	0.40	0.45	**0.007**	**0.001**	**0.001**

Table 76. Influence of cholera vaccination (CH#54 and CH#55) on the proportion of each short chain fatty acid of total short chain fatty acids (C2 – C7) in ileal and caecal digesta of SPF leghorns at 76 d old fed continuously on unsterilised commercial starter and grower diets.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Control	♀	7[2]	**1.000**	**0**	0	**0**	0	0	0	0	N/A
	Control	♂	7[2]	**1.000**	**0**	0	**0**	0	0	0	0	
	CH#54	♀	7[2]	**0.968**	**0.002**	0.031	**0**	0	0	0	0	
	CH#54	♂	8[1]	**0.912**	**0.023**	0.037	**0.007**	0.006	0.004	0.004	0.007	
	SE[2]			0.0195	0.0050	0.0119	—	—	—	—	—	
	SE[1]			0.0195	0.0050	0.0119	0.0013	0.0023	0.0020	0.0021	0.0022	
	P			**0.011**	**0.007**	0.07	**0.001**	0.24	0.41	0.41	0.07	
Ileum	Control	♀	7[2]	0.971	0.009	0.008	0.006	0	0.001	0.003	0.001	N/A
	Control	♂	8[1]	0.993	0.006	0.001	0.001	0	0	0	0	
	CH#55	♀	7[2]	0.994	0.006	0	0	0	0	0	0	
	CH#55	♂	8[1]	0.991	0.001	0.009	0	0	0	0	0	
	SE[2]			0.0087	0.0048	0.0056	0.0018	—	0.0004	0.0012	0.0005	
	SE[1]			0.0081	0.0045	0.0053	0.0017	—	—	—	—	
	P			0.20	0.62	0.54	0.053	—	0.36	0.13	0.12	
Caeca	Control	♀	7[2]	0.776	0.030	**0.174**	**0.008**	0.009	0.003	<0.001	0	N/A
	Control	♂	7[2]	0.730	0.034	**0.217**	**0.005**	0.012	0.002	<0.001	tr	
	CH#54	♀	7[2]	0.720	0.032	**0.232**	**0.005**	0.010	0.002	0	tr	
	CH#54	♂	8[1]	0.731	0.025	**0.232**	**0.004**	0.008	0.001	<0.001	0	
	SE[2]			0.0174	0.0040	0.0157	0.0005	0.0017	0.0005	0.0001	—	
	SE[1]			0.0163	0.0038	0.0146	0.0004	0.0016	0.0004	0.0001	—	
	P			0.13	0.39	**0.045**	**0.001**	0.35	0.33	0.81	—	
Caeca	Control	♀	7[2]	0.774	0.027	0.161	0.009	0.012	0.007	**0.005**	**0.005**	N/A
	Control	♂	8[1]	0.789	0.040	0.151	0.004	0.010	0.004	**0.001**	**0**	
	CH#55	♀	7[2]	0.702	0.031	0.229	0.010	0.017	0.009	**0.001**	**0**	
	CH#55	♂	8[1]	0.773	0.040	0.167	0.006	0.010	0.005	**0.001**	**0**	
	SE[2]			0.0326	0.0055	0.0311	0.0019	0.0030	0.0027	0.0009	0.0009	
	SE[1]			0.0305	0.0052	0.0291	0.0017	0.0028	0.0026	0.0008	—	
	P			0.25	0.27	0.29	0.11	0.32	0.45	**0.001**	**0.001**	

Trial 6

Digesta and plasma L- and D-lactic acid concentrations (Table 77) were unaltered ($p > 0.05$) by vaccination in the first trial (Trial 6a) but males produced more ($p < 0.05$) ileal L- and D-lactic acid than females. Cholera vaccination lowered ($p < 0.05$) ileal D-lactic acid in the second trial (Trial 6b).

Table 77. Influence of cholera vaccination (CHO#4053-001) on the digesta and plasma L- and D-lactic acid concentration (mMol/l) of SPF leghorns at 65 and 71 d old fed continuously on an irradiated commercial starter diet.

Vaccine	Sex	N	Ileum		Caeca		Plasma	
			L-lactic	D-lactic	L-lactic	D-lactic	L-lactic	D-lactic
Control	♀	5	37.22	0.38	0.61	0.78	3.68	0
Control	♂	5	29.98	0.87	0.52	0.67	3.65	0
CHO#4053	♀	5	32.23	0.26	0.63	0.86	3.97	0
CHO#4053	♂	5	38.27	0.45	0.50	0.59	4.52	0
SE			9.374	0.246	0.055	0.077	0.466	—
P			0.91	0.35	0.30	0.12	0.54	—
Control	♀	6[1]	**32.80**^{y}	**0.77**^{ay}	6.75	1.49	5.27	0
Control	♂	4[2]	**47.32**^{x}	**1.65**^{ax}	10.71	1.28	6.79	0
CHO#4053	♀	4[2]	**42.45**^{y}	**0.64**^{by}	10.58	1.42	5.20	0
CHO#4053	♂	4[2]	**54.03**^{x}	**0.88**^{bx}	13.54	1.22	5.61	0
SE[1]			4.390	0.178	2.124	0.104	0.535	—
SE[2]			5.376	0.218	2.601	0.128	0.655	—
P			**0.047**	**0.021**	0.27	0.36	0.28	—

Total anaerobe, aerobe and MRS (lactic acid bacteria) counts did not differ ($p > 0.05$) across the ileum and caeca in Trial 6a nor ileal MRS counts in Trial 6b (Table 78).

Table 78. Influence of cholera vaccination (CHO#4053-001) on the ileal and caecal digesta microbial counts (cfu ml^{-1}) of SPF leghorns at 65 and 71 d old fed continuously on an irradiated commercial starter diet.

Gut	Count	Microbial counts (cfu x 10^9) per ml of digesta					
		Control		CHO#4053			
		♀	♂	♀	♂	SE	p
Ileum	Total anaerobes	1.14	13.39	1.37	0.87	4.282 x 10^9	0.45
	Total aerobes	1.82	1.92	1.31	0.97	5.831 x 10^8	0.92
	Lactic acid bacteria	1.46	1.32	0.94	0.84	3.999 x 10^8	0.64
Caeca	Total anaerobes	1.33	1.14	1.61	1.86	4.282 x 10^9	0.45
	Total aerobes	1.41	1.28	1.68	1.93	5.831 x 10^8	0.92
	Lactic acid bacteria	0.67	0.57	0.65	0.57	3.999 x 10^8	0.64
Ileum	Lactic acid bacteria	7.63	3.78	9.18	8.31	1.677 x 10^9	0.099*

*log count probability

In the ileal digesta in Trial 6a, *n*-butyric and heptanoic acid concentrations (mMol/L) (Table 79) were higher ($p < 0.05$) in unvaccinated females and males respectively than other birds. Males had higher ($p < 0.05$) concentrations of *iso*-butyric acid than females. In Trial 6b *iso*-butyric and heptanoic acid concentrations were reduced ($p < 0.05$) in vaccinated birds. In caecal digesta, in both trials *iso*-butyric acid was greater ($p < 0.05$) in vaccinated birds and males. Additionally, in Trial 6b, *n*-butyric acid concentration was reduced ($p < 0.05$) in vaccinated birds and increased in females while *iso*-valeric acid was greater ($p < 0.05$) in vaccinated birds.

In Trial 6a, ileal digesta proportions of *n*-butyric acid (Table 80) were greater ($p < 0.05$) in unvaccinated females than males or vaccinated birds; vaccinated females had more ($p < 0.05$) *iso*-butyric acid than

vaccinated males or unvaccinated females but similar ($P > 0.05$) proportions to unvaccinated males. Heptanoic acid proportions were greater ($p < 0.05$) in unvaccinated males than unvaccinated females or vaccinated males but similar ($p > 0.05$) to those in vaccinated females. In Trial 6b, ileal digesta had a lower ($p < 0.05$) proportion of *iso*-butyric acid in vaccinated birds and unvaccinated females had more ($p < 0.05$) heptanoic acid than males or vaccinated birds. Caecal digesta VFA proportions were unaltered ($p > 0.05$) in Trial 6a and only *n*-valeric acid was in greater ($p < 0.05$) proportion in females than males in Trial 6b.

Two additional sets of samples had VFA contents examined. In Trial 6a, colonic digesta had similar ($p < 0.05$) concentrations and proportions (Table 81) of VFA's across both sex and vaccination administration. In Trial 6b, serum concentrations of acetic, *iso*-butyric and total acids were lower ($p < 0.05$) in vaccinated birds and a reduced ($p < 0.05$) proportion (Table 81) of *iso*-butyric acid was found in the serum of vaccinated birds.

Table 79. Influence of cholera vaccination (CHO#4053-001) on the concentration (mMol/L) of short chain fatty acids (C2-C7) in the ileal and caecal digesta of SPF leghorns at 65 and 71 d old fed continuously on an irradiated commercial starter diet.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Control	♀	5	11.968	0.075	**0.263**	**0.763**	0	0	0.006	**0.027**	13.102
Concentration	Control	♂	5	7.540	0.099	**0**	**1.050**	0	0	0.006	**0.160**	8.848
Trial 1 65 d	CHO#4053	♀	5	5.577	0.017	**0**	**1.005**	0	0	0	**0.065**	6.672
	CHO#4053	♂	5	10.250	0.200	**0**	**1.079**	0.008	0	0.0306	**0**	11.542
	SE			2.1001	0.0704	0.0614	0.0785	0.0038	—	0.0052	0.0268	2.1245
	P			0.18	0.35	**0.017**	**0.044**	0.42	—	0.80	**0.004**	0.19
Ileum	Control	♀	6[1]	16.755	0.602	0.008	**0.389**	0.005	0	0	**0.230**	17.988
Concentration	Control	♂	4[2]	24.838	1.375	0.366	**0.383**	0	0	0	**0.102**	27.064
Trial 2 71 d	CHO#4053	♀	5[3]	18.596	0.606	0.089	**0.180**	0	0	0.011	**0.072**	19.554
	CHO#4053	♂	5[3]	17.469	2.070	0.230	**0.215**	0	0.018	0	**0.047**	20.050
	SE[1]			3.1612	0.7353	0.1359	0.0313	0.0029	—	—	0.0363	3.5350
	SE[2]			3.8716	0.9005	0.1665	0.0384	—	—	—	0.0445	4.3295
	SE[3]			3.4629	0.8055	0.1489	0.0343	—	0.0090	0.0053	0.0398	3.8724
	P			0.42	0.52	0.39	**0.001**	0.54	0.42	0.42	**0.015**	0.44
Caeca	Control	♀	5	74.704	1.091	10.805	**0.226**	1.212	0.345	0.038	0.071	88.492
Concentration	Control	♂	5	81.211	1.038	6.956	**0.384**	1.092	0.394	0	0.015	91.090
Trial 1 65 d	CHO#4053	♀	5	80.786	1.071	7.833	**0.469**	0.915	0.342	0.006	0	91.422
	CHO#4053	♂	5	93.474	1.272	14.047	**0.600**	1.478	0.395	0	0	111.266
	SE			13.0148	0.2076	2.7685	0.0466	0.2338	0.0712	0.0158	0.0235	8.3292
	P			0.78	0.86	0.29	**0.001**	0.41	0.91	0.30	0.14	0.70
Caeca	Control	♀	6[1]	20.471	2.101	7.724	**0.136**	**0.177**	**0.155**	0.025	**0.088**	30.876
Concentration	Control	♂	4[2]	20.797	1.096	5.845	**0.256**	**0.048**	**0.204**	0	**0.165**	28.414
Trial 2 71 d	CHO#4053	♀	5[3]	22.744	2.127	9.504	**0.294**	**0.093**	**0.258**	0	**0.238**	35.259
	CHO#4053	♂	4[2]	26.531	2.387	9.690	**0.384**	**0**	**0.241**	0	**0.246**	39.476
	SE[1]			3.6750	0.4797	1.6235	0.0346	0.0228	0.0201	0.0145	0.0382	5.4180
	SE[2]			4.5009	0.5875	1.9884	0.0423	0.0280	0.0246	—	0.0468	6.6357
	SE[3]			4.0257	0.5255	1.7785	0.0378	0.0250	0.0220	—	0.0419	5.9351
	P			0.74	0.44	0.48	**0.003**	**0.001**	**0.016**	0.58	**0.048**	0.64

Table 80. Influence of cholera vaccination (CHO#4053-001) on the proportion of each short chain fatty acid of total short chain fatty acids (C2 – C7) in ileal and caecal digesta of SPF leghorns at 65 and 71 d old fed continuously on an irradiated commercial starter diet.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Control	♀	5	0.897	0.007	**0.026**	**0.070**	0	0	0.001	**0.001**	N/A
Concentration	Control	♂	5	0.837	0.009	**0**	**0.134**	0	0	0	**0.020**	
Trial 1 65 d	CHO#4053	♀	5	0.801	0.002	**0**	**0.179**	0	0	0.002	**0.160**	
	CHO#4053	♂	5	0.880	0.015	**0**	**0.103**	0.001	0	0.001	**0**	
	SE			0.0291	0.0058	0.0072	0.0249	0.0006	—	0.0010	0.0057	
	P			0.13	0.45	**0.014***	**0.041**	0.42	—	0.71	**0.046**	
Ileum	Control	♀	6[1]	0.929	0.035	0.001	**0.023**	<0.001	0	0	**0.013**	N/A
Concentration	Control	♂	4[2]	0.924	0.043	0.010	**0.018**	0	0	0	**0.005**	
Trial 2 71 d	CHO#4053	♀	5[3]	0.948	0.034	0.004	**0.010**	0	0	0.001	**0.003**	
	CHO#4053	♂	5[3]	0.890	0.086	0.010	**0.011**	0	0.001	0	**0.002**	
	SE[1]			0.0277	0.0236	0.0040	0.0028	0.0002	—	—	0.0016	
	SE[2]			0.0339	0.0289	0.0049	0.0034	—	—	—	0.0019	
	SE[3]			0.0303	0.0259	0.0044	0.0031	—	0.0003	0.0006	0.0017	
	P			0.61	0.45	0.35	**0.028**	0.54	0.42	0.42	**0.001**	
Caeca	Control	♀	5	0.841	0.013	0.122	0.003	0.015	0.005	0.001	0.001	N/A
Concentration	Control	♂	5	0.895	0.012	0.072	0.004	0.012	0.005	0	<0.001	
Trial 1 65 d	CHO#4053	♀	5	0.879	0.011	0.089	0.006	0.011	0.004	0	0	
	CHO#4053	♂	5	0.843	0.011	0.122	0.006	0.013	0.004	0	0	
	SE			0.0209	0.0017	0.0190	0.0008	0.0022	0.0013	0.0003	0.0003	
	P			0.22	0.89	0.20	0.08	0.65	0.98	0.42	0.15	
Caeca	Control	♀	6[1]	0.662	0.070	0.244	0.005	**0.008**	0.006	0.002	0.004	N/A
Concentration	Control	♂	4[2]	0.757	0.032	0.189	0.010	**0.002**	0.008	0	0.006	
Trial 2 71 d	CHO#4053	♀	5[3]	0.652	0.055	0.267	0.009	**0.002**	0.008	0	0.008	
	CHO#4053	♂	4[2]	0.662	0.062	0.251	0.010	**0**	0.008	0	0.007	
	SE[1]			0.0321	0.0105	0.0274	0.0014	0.0014	0.0013	0.0009	0.0017	
	SE[2]			0.0393	0.0128	0.0335	0.0017	0.0017	0.0015	—	0.0021	
	SE[3]			0.0352	0.0115	0.0300	0.0015	0.0016	0.0014	—	0.0019	
	P			0.22	0.17	0.37	0.13	**0.019**	0.57	0.58	0.41	

*log transformed data probability

Table 81. Influence of cholera vaccination (CHO#4053-001) on the concentration (mMol/L) and proportion of short chain fatty acids (C2 – C7) in colonic digesta of SPF leghorns at 65 d old and serum of SPF leghorns at 71 d old fed continuously on an irradiated commercial starter diet.

Gut section	Vaccination	Sex	n	Short chain fatty acid (C2-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Colon	Control	♀	4[1]	43.950	0.543	7.155	0.709	0.589	0.047	0	0.029	53.022
Concentration	Control	♂	2[2]	53.239	1.201	9.851	0.559	0.882	0.137	0	0.082	65.950
Trial 1 65 d	CHO#4053	♀	4[1]	44.775	0.509	5.876	0.440	0.479	0.034	0	0	52.111
	CHO#4053	♂	5[3]	37.664	0.529	4.820	0.527	0.262	0	0	0.068	43.871
	SE[1]			11.7311	0.2020	2.2098	0.0665	0.2136	0.0420	—	0.0263	14.2897
	SE[2]			16.5903	0.2857	3.1252	0.0940	0.3020	0.0593	—	0.0373	20.2087
	SE[3]			10.4926	0.1807	1.9765	0.0595	0.1910	—	—	0.0236	12.7811
	P			0.88	0.24	0.58	0.09	0.39	0.33	—	0.22	0.83
Colon	Control	♀	4[1]	0.835	0.008	0.109	0.038	0.008	0.001	0	0.001	N/A
Proportion	Control	♂	2[2]	0.807	0.017	0.151	0.010	0.013	0.002	0	0.002	
Trial 1 65 d	CHO#4053	♀	4[1]	0.874	0.009	0.098	0.011	0.007	0.001	0	0	
	CHO#4053	♂	5[3]	0.866	0.014	0.098	0.015	0.005	0	0	0.002	
	SE[1]			0.0156	0.0025	0.0228	0.0149	0.0023	0.0005	—	0.0007	
	SE[2]			0.0221	0.0035	0.0322	0.0219	0.0032	0.0007	—	0.0010	
	SE[3]			0.0140	0.0022	0.0204	0.0133	0.0021	—	—	0.0007	
	P			0.09	0.14	0.56	0.54	0.25	0.38	—	0.28	
Serum	Control	♀	6[1]	**1.331**	0.059	0	**0.127**	0.004	0	0.007	0.051	**1.578**
Concentration	Control	♂	4[2]	**1.163**	0	0	**0.098**	0	0	0	0.091	**1.352**
Trial 2 71 d	CHO#4053	♀	5[3]	**0.789**	0.050	0	**0.027**	0	0	0.008	0.027	**0.901**
	CHO#4053	♂	5[3]	**0.710**	0	0	**0.010**	0	0	0	0.027	**0.747**
	SE[1]			0.1150	0.0252	—	0.0118	0.0021	—	0.0052	0.0316	0.1274
	SE[2]			0.1408	—	—	0.0144	—	—	—	0.0387	0.1560
	SE[3]			0.1260	0.0276	—	0.0129	—	—	0.0057	0.0346	0.1396
	P			**0.007**	0.30	—	**0.001**	0.54	—	0.66	0.58	**0.002**
Serum	Control	♀	6[1]	0.839	0.034	0	**0.083**	0.002	0	0.006	0.036	N/A
Proportion	Control	♂	4[2]	0.861	0	0	**0.072**	0	0	0	0.068	
Trial 2 71 d	CHO#4053	♀	5[3]	0.888	0.048	0	**0.027**	0	0	0.011	0.026	
	CHO#4053	♂	5[3]	0.949	0	0	**0.015**	0	0	0	0.036	
	SE[1]			0.0364	0.0180	—	0.0104	0.0012	—	0.0060	0.0286	
	SE[2]			0.0445	—	—	0.0127	—	—	—	0.0351	
	SE[3]			0.0398	0.0197	—	0.0114	—	—	0.0065	0.0314	
	P			0.25	0.26	—	**0.001**	0.54	—	0.61	0.83	

The proportions of fatty acid methyl esters in the ileal mucosa (Table 82) was unaltered ($p > 0.05$) by cholera vaccination.

Table 82. Influence of cholera vaccination (CHO#4053-001) on the fatty acid methyl esters (% total fatty acid) in the ileal mucosa of SPF leghorns at 65 d old fed continuously on an irradiated commercial starter diet.

Fatty acid	Control		CHO#4053-001		SE	
	♀ [a]	♂ [a]	♀ [b]	♂ [a]	n=5 [a]	n=4 [b]
C8:0	0	0	0	0	0	0
C10:0	0	0	0	0	0	0
C14:0	0.23	0.24	0.27	0.23	0.041	0.045
C14:1n-7	0.34	0.24	0.28	0.40	0.069	0.077
C16:0	16.42	15.55	15.92	14.90	0.738	0.825
C16:1n-7	0.51	0.53	0.47	0.60	0.106	0.119
C18:0	17.62	18.28	16.10	16.71	1.325	1.482
C18:1n-9	19.87	16.98	19.84	16.37	1.227	1.372
C18:1n-7	1.77	1.69	1.69	1.60	0.092	0.103
C18:2n-6	22.48	22.35	21.56	19.68	1.320	1.476
C18:3n-6	0.56	0.68	0.53	0.59	0.063	0.070
C18:3n-3	0.37	0.45	0.43	0.46	0.073	0.082
C20:0	0.61	0.72	0.70	0.63	0.088	0.098
C20:1n-9	0.23	0.22	0.23	0.35	0.047	0.053
C20:2n-6	0.17	0.20	0.23	0.21	0.047	0.053
C20:3n-6	0.60	0.70	0.79	0.82	0.098	0.110
C20:4n-6	6.42	6.80	6.52	6.27	0.347	0.387
C20:5n-3	2.51	3.22	2.10	2.73	0.362	0.405
C22:0	0.30	0.30	0.34	0.30	0.044	0.050
C22:1n-9	0	0	0	0.05	0.025	0
C22:2n-6	0	0	0.10	0.07	0.035	0.040
C22:3n-3	0.22	0.31	0.28	0.28	0.057	0.063
C22:5n-3	0.20	0.24	0.24	0.30	0.057	0.064
C22:6n-3	2.71	2.36	1.79	3.32	0.374	0.418
C24:0	0.47	6.58	9.54	11.11	2.888	3.229
C24:1n-9	5.61	1.37	0.06	2.04	2.017	2.256

Plasma myeloperoxidase concentrations in all birds were below detection limits in the first sample set. In the second set, the second replicate trial of cholera vaccine CHO#4053-001, two vaccinated female birds returned low (3.85 ng ml^{-1}) and moderate (13.81 ng ml^{-1}) MPO concentrations. All other birds returned levels below detection limits.

8.4 Discussion

Fowl cholera is not mentioned in the European Pharmacopeia and the vaccines used in these experiments (and in the field) were produced under permit i.e. they were not registered. A stable seedlot is needed for registrable vaccines but the point of fowl cholera vaccine is to move with the change in the organism in the field: registration thereby being precluded. The different vaccines used, denoted by different isolate (or isolate combinations) designations, adjust for surface antigen change to keep up with the field biology of the organism.

The series of experiments conducted for this work indicated that some changes in digesta pH could be replicated across a range of cholera types and that a reduced ileal digesta pH was common when individual vaccines were given, when two different types were tested simultaneously and, in the final experiments, when the same type was given to successive groups of birds. Some changes in ileal and caecal SCFA concentrations and proportions were noted and which were altered across sex consistently. In particular, the propensity for greater acetic, and therefore total, acid concentrations to be produced in males was indicated. L- and D-lactic acid concentrations in ileal digesta were found across males or vaccine in the final trial. Digesta microbial counts, ileal mucosal fatty acid constituents and myeloperoxidase concentrations provided little evidence of gross changes in hindgut function however. The limited resources available to the work did not allow for substantial replication to pursue the effects of changes in the digesta on gut function.

Given the limitations of these studies, as changes in the markers varied across the trials reported here, the first hypothesis, that of an effect of the adjuvant may be rejected: the adjuvant should have caused similar responses. The second hypothesis may have been supported by differences in the fermentation markers being found when the various vaccines were applied. However, as some variations in the differences were

found with replicated use of the one vaccine (Experiment 7), further work is required to examine the effects of vaccines upon gut function. It is further suggested that any work could better be commenced using a stable organism (vaccine) rather than fowl cholera. Also, the effect of gut-organism based adjuvants should be pursued in relation to the effects of vaccination upon gut function and their potential to precipitate changes in gut function leading to bouts of enteric disease.

9. General Discussion

The current project developed from data supporting the hypothesis that dietary cereal change created, in the short term, a fermentative or lactic acidosis in the hindgut of layer stock. Consistency of the response was found across a range of cereals including "good" and "bad" wheat and rice compared with the commercial ration that the birds had been fed through their respective starter, grower and layer phases. This response was greater with a highly fermentable grain, rice, with its minimal content of structural carbohydrate and with minimal processing effects such as steam-pelleting. These effects were reproduced in broilers at various points in the early and later starter phases. Importantly, the experiment in which a range of cereals were fed from day-old produced no substantial, significant production of fresh blood and mucus in the excreta; those symptoms that are closely associated with inflammatory bowel disease/ulcerative colitis in a range of animal models and under different conditions. As some evidence of hindgut tissue inflammation was revealed through commercial histopathological analysis, diet change alone can have untoward effects on the hindgut tissue and must be considered for its potential to allow bouts of enteric disease to occur in poultry.

Changes in excreta pH were found in both short and long term trials which confirmed the results of earlier work (Taylor, 2002). Digesta pH change was as found earlier, with either maintenance of the same pH, despite reductions in excreta pH, or an increase in ileal pH. Increases in excreta pH were reported in the work of Clayton and Buffinton (2000) who created colitis in mice and recorded subsequent blood loss in the excreta and diarrhoea. The pH increase was suggested as being from the greater mucus secretion following acidosis. Roediger *et al*. (1984) reported that in human ulcerative colitis a low colonic lumen pH was due to a failure of bicarbonate secretion from damaged tissue. In the later SPF bird trials, changes in the dietary cereal resulted in reductions in ileal digesta pH after 5 d. This may have been associated with greater organic acid concentration. However, of the organic acids, the SCFA concentrations were largely unaltered but lactic acid concentrations increased. In the rat ileum and colon, Saunders and Sillery (1982) showed that high H^+ concentrations and lactate caused a reduction of net water transport and cell loss increased.

Lactic acid was foremost amongst the organic acid changes found in the birds upon feed changes being made. The rice-based diets resulted in the greatest concentrations of lactic acid being produced, and in considering just the D- isomer alone, its concentration was at levels denoting at least "light" acidosis in the sheep ileum (Ding and Xu, 2003). To this must be added the greater concentration of the L-isomer. Through the layer, broiler and SPF bird trials, ileal lactic acid concentration was increased following dietary change. In a broiler trail in which different cereals were fed from day old, moderation of changes in pH and organic acids followed and which did not create differences in blood being lost in the excreta. Short chain fatty acids were little altered in the short term. However, when longer periods of feeding were allowed after dietary change, in the later SPF birds trials, alterations in various of the SCFA concentrations were found. Largely this was attributed to reductions in acetic acid concentrations and its proportion of total acid. This was found in the caeca but to a lesser degree in the distal ileum.

Kim and Berstad (1992) reviewed the range of animal models used to study gut inflammation in humans and concluded that the development of inflammatory bowel diseases, the correct therapeutic approach to treatment and the ideal animal model were all unknown. Many substances and dietary manipulations have been employed to create inflammation but no model is yet regarded as stable and reproducible (Pacheco *et al*., 2000). Many models have used an acetate instillation (Sharon and Stenson, 1985; Fabia *et al*., 1994) which produces many symptoms including those found in the ileal tissue in the birds in these experiments. Sharon and Stenson (1985) suggested that the arachidonic acid metabolism of acetate colitis and IBD indicated that despite a range of stimuli, inflammatory responses in the intestine are all modulated by the same soluble mediators, and, thus, similarities among the intestinal inflammatory diseases could be explained.

The complex interactions of diet, gut microbes and health have been long studied and were highlighted by Metchnicoff at the end of the 19th century. In 1981, Wolin reviewed the study of the human intestine for its complex interactions of diet, microbial populations and health and noted that the rumen and the human colon were functionally the same in both fermentation patterns and products. These ideas have been intensively studied and have taken many complex paths, many of which have lead to much confusion and, patently, little understanding of gut function. Furthermore, many research programmes have been subject

to, or directed at, specific commercial outcomes. Research into gut function has been of intense interest to the poultry industry because of the needs of ever greater production efficiency, some advances in feed efficiency with application of exogenous feed enzymes and more recently with the imminent removal of most of the remaining antimicrobial products available for widespread use.

The application of two products, virginiamycin and avilamycin, under different conditions, showed that from day old, virginiamycin altered some factor in the gut that changed the, albeit minimal, blood loss in the excreta of young broilers to 21 days of age. Avilamycin did not prevent the development of blood loss in excreta from or diarrhoea in older broilers fed a known "problem" wheat (HP S1).

The object of the current trials was to focus on the possibility that many enteric disease breaks could be precipitated by sudden changes to the cereal base of the diet. There is evidence that the fermentation pattern established in the birds' gut could suffer a perturbation that, however brief or mild, could lead to greater immediate problems or later in the production cycle.

Changes in dietary fat content and type will alter the fatty acid composition of the gut. With high rates of gut cell turnover this may be accelerated and place demands upon requirements for particular fatty acids. For example, jejunal enterocyte membrane phospholipid biogenesis needs rapid provision of the essential polyunsaturated fatty acids which are only available from the diet (Garg *et al*., 1997). The fatty acid composition of membrane phospholipids is important in determining the uptake of nutrients as well as preventing inflammatory disease and the gastrointestinal tract must be recognized for its importance as a primary barrier between the external and internal environments (Garg *et al*., 1997).

The suggestions of mucosal irritation or inflammation in both the distal ileum and colo-rectum, provided by commercial histopathology, stemmed from, or were at least associated with, modest and often short-lived reductions in pH and increases in digesta lactic acid but with minor, if any, alterations in SCFA levels. In considering this inflammation, the measurement of myeloperoxidase was undertaken. The chicken was believed to be deficient in MPO (Ferencik *et al*., 1976) due to the differences in some types of polymorphonuclear leucocytes between species (Dri *et al*., 1978). However, Lam (1997) showed that chicken heterophils contain a DNA sequence homologous to segment 10 of the human MPO gene and further assay demonstrated MPO activity. Recently, Quindry *et al*. (2003) measured MPO in the plasma of athletes undergoing heavy exercise. The application of the method to chickens provided few positive results, as Quindry *et al*. (2003) found that the direct enumeration of neutrophils in the blood was more reliable than MPO level, perhaps heterophil proportions would be a useful measure in chickens.

Practical considerations

The various strands of this project suggest that an extremely conservative approach to feed formulation and changes to cereal components of diets for all classes of chickens is required to minimise or prevent perturbations in hindgut function. Modest changes to diet can interfere with fermentative function through disturbance of the rate of substrate passage, alterations in substrate types and consequent alterations of hindgut microbial populations. Despite some gross effects, such as digesta pH, being transitory, substantial evidence of direct inflammatory effects upon hindgut mucosae, particularly in the ileum was found. Together with some more subtle effects, such as upon mucosal fatty acid composition, the blood and mucus losses from the hindgut do not augur well for stability of the hindgut environment and suggest that some of the problems of enteric disease which beset poultry production may be triggered by dietary change. These effects require larger scale, longer term study to determine their extent and general application to the field.

With much interest in the development of gut delivery of vaccines, the results of the cholera trials indicate that systemic effects of vaccines and, importantly, some forms of adjuvants, should be considered for effects upon the gut environment. Substantial changes in digesta pH, organic acid concentrations and proportions, associated with body weight checks certainly suggest that adjuvant contents, particularly of microbial origin, need to be considered for effects on bird production. Furthermore, a general strengthening of vaccine testing and commercial data presentation should be provided to the poultry industry; the international standards, via many a manual/pharmacopoeia, do not require the detail as was once used by an Australian vaccine manufacturer.

A major conclusion from the current work is that scientific methods employed in pursuing gut inflammation could adopt a diet-based method of creating mucosal lesions. A common criticism of animal

models of inflammatory bowel disease, either ulcerative colitis or Crohn's disease, is that many if not most of the range of gut irritants used do not produce consistent responses in any of the animal models. At least in poultry, whether layer or broiler birds, a consistent production of "colitis-like" symptoms – fresh blood and mucus – was achieved following substitution of the birds' stable, commercial diet, with one based upon another cereal. This was greater when a rice diet was fed to layer or broiler stock. Gut inflammation was noted in both the ileum (hence the suggestion of the term "ileitis" being applied) and colo-rectum. These findings suggest that the development of the more problematic poultry enteric diseases could be studied over the course of the production cycle following dietary disturbances.

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