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& DEVELOPMENT CORPORATION

Hindgut function in laying hens

A report for the Rural Industries Research and Development Corporation

by Robert Taylor

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Foreword

The efficiency of cereal grain conversion to poultry products has involved focus on the nutritive effects of the constituents of the various cereals. Concurrent with the application of exogenous feed enzymes and other products which aid in the digestive process, has been much recent work to determine the effects of individual grain constituents and how these fractions may be influenced by growing, storage and processing conditions.

A considerable research effort has been devoted to attempts to accurately predict the feeding value of a particular cereal to allow for effective feed formulation. This effort has covered the range of livestock industries with ever more co-ordinated research programs. Collaboration with human nutritionists has been useful particularly for identifying negative effects, including nutrition-disease interactions, that may be directly linked with cereal consumption.

One aspect of this work that has been little considered in poultry is the potential effect of an acidosis due to fermentation of a large carbohydrate load. This may be of concern with abrupt substitution of one cereal type with another, grains from different growing location or, simply, storage time (the "new season" grain phenomenon which, commercially, had been long recognised by the major integrators).

This report details a series of introductory experiments designed to provide evidence of a fermentative acidosis caused in the hindgut gut of layer-type birds after abrupt changes in dietary cereal type, grain processing or feeding methods and application of a commercial feed enzyme.

This project was funded by the Egg Industry R&D Corporation from industry revenue which is matched by funds provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 450 research publications, forms part of our Egg industry 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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Peter Core
Managing Director
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Contents

Executive Summary

Intake of a large carbohydrate load can cause serious problems in many animals due to an increase in the concentration of the products of fermentation which the metabolic processes of the animal has difficulty assimilating. This is well recognised in ruminant animals suddenly introduced to grain and is now being considered more in monogastric animals including pigs and humans. There are many types of carbohydrate fractions in the different cereals it can be the interactions of these constituents and what the animals gut and/or its attendant micro-organism load is adapted to processing that may lead to problems.

In an effort to determine if a fermentative acidosis can develop in the lower gut of layer-type birds, a series of simple trials presented the birds with sudden changes to the cereal base of the diet, alterations to the method of feed processing and feeding, or the inclusion of a commercial enzyme. This acidosis was to be measured by the levels of organic acids (the short-chain fatty and lactic acids) found in the lower intestine, the caeca and the short colon of the birds after single-cereal diets were fed to the birds.

Feeds were formulated to include as much of a single cereal as possible. The feeds were based on recommended specifications for the type of birds used in the trials and ingredients were those included in commercial diets. Wheat, sorghum, barley and rice diets were used.

Initially, three broiler trials were conducted to provide for development, testing and/or validation of the sampling and analytical methods. Additionally, these trials allowed for some further examination of grain processing and its effects on digesta throughout the gut. Production data such as body weight gain and feed conversion efficiency were reported elsewhere (Jones and Taylor, 2001) and some differences were found with the different cereals when they were included as 20% whole grain in the feed mix prior to pelleting. The general response was for an early moderation of weight gain and poorer feed conversion followed by a compensatory period in the later growing phase. Perhaps more importantly, with all cereals, there was a reduction in the incidence of proventricular dilatation and a reduced risk of death due to ascites.

The broiler trials highlighted the generally good quality of commercially available feed grain over the two seasons (grain procured from February 2000 to Feb 2001); a point made by both feed company and consultant nutritionsts and several researchers alike. Results from commercial enzyme inclusion in the diets were nevertheless significant at times and improvements in several specific measurements that define the point of enzyme application occurred. These included reductions in digesta viscosity when a full wheat diet was used and digesta pH was often higher; into the range of optimal pH for the starch digesting enzyme (amylase) produced by the bird, gut micro-organisms or the cereals themselves. The concentration of microbial fermentation products in the lower gut appeared to be greater when enzyme was added to the diets. This is of some interest in indirectly monitoring gut microbe populations and activity but is of dubious productive value to the bird. Furthermore, it leads to some questioning of the value of some current research methods used to evaluate energy availability. This is especially so when whole grain inclusion produced some similar responses to the application of feed enzymes.

Two separate groups of the same commercial-cross laying bird were used over the course of the experiments and the different feeds were trialed at various points in the growing and laying phases of the production cycle.

Over the course of all layer bird experiments the results were, at times, contradictory. There were several consistent results that emerged. It was apparent that pH of the digesta throughout the tract changed quite dramatically over the course of the day. This may be explained by the laying bird eating its feed in the light period and, with these birds, having a relatively large “meal” after the lights were switched on, and with the digesta clearing the gut over the course of the day. There was a

trend for the pH in the lower gut to be lower after 12 h exposure to the feed and this was sometimes found at 36 h. Conversely excreta pH and that in the digesta in the lower small intestine and colon was quite high (> 7.5) at 24 or 48 h when the birds had eaten only 1-3 h prior to being euthanased. The pH of contents in the upper tract, the crop and gizzard, altered dramatically with the volume of feed and time after feeding.

Plasma pH, which is maintained within strict limits, consistently decreased over the 48 h of the experiments and this could provide a subtle indication that a general acidotic condition, due to fermentation products being absorbed and metabolised, whilst not measurable in great quantity at any one point, was having an effect on the buffering capacity (the capacity to deal with changes in H ion concentration; acid in effect) of the body fluids. A simple alternative is that repeated blood sampling and the associated stress was interfering with fluid levels and/or affected normal respiratory function in the longer term.

The different cereals provided varying responses at times and it was of note that rice caused some significant declines in pH and produced significant concentrations of both the fatty acids and lactic acid. Rice has little of the more complex or structural carbohydrates such as the non-starch polysaccharides which are believed to cause many of the problems of poor utilisation by poultry at times. The starch is more readily exposed to the digestive process. However, this is derived from results from cooked rice. The cooking of any starch changes its structure to allow ready breakdown by digestive enzymes (whether they be those of the grain itself, the animal or the microbes). However, starch is present in different forms and may not be quickly accessible in the birds gut. The native starches are resistant to an animals amylase to a greater or lesser extent. The finding of similar concentrations of the L- and D- forms of lactic acid in the ileum or caeca of birds fed rice but not wheat or barley may indicate a problem. Although the lactic acid may be little absorbed, there are other effects on the mucosa by its accumulation and in association with the total organic acid concentration of fermentation products.

A second group of birds was grown on feed sourced from a different feed miller. In comparisons of this commercial feed with the then current seasons, though aged, wheat, indicated an increase in fermentative activity. At times, the wheat diet produced high concentrations of lactic acid (the D-isomer being of concern) and VFA levels were similarly increased. A different year and grain source provided results that suggested that the digestion of the feed and, one presumes, the starch component was altered. There was greater fermentation occurring in the distal ileum or caeca.

From a practical viewpoint there was, overall, some evidence of a fermentative or lactic acidosis generally caused by sudden changes in diet type or processing. There were small changes that may, over time, lead to negative effects in the gut. This may be as simple as the preponderance of acetic acid being produced as it has, in strong concentration, been shown to cause cellular damage to the gut in several species of animals. Indeed this has provided a model for induction of serious disease conditions of the gut in animals such as mice used for human medical research. In the bird, it is proposed that a mild concentration may induce damage if it is maintained over a long period. The gut damage could then impact upon production responses or provide the opportunity for disease challenge.

A consistent finding throughout the course of the experiments was that a sudden feed change initiated several changes in excreta conditions that were symptomatic of an immune response. This led to successful application to continue this line of research.

1. Introduction

1.1 Background to proposal

With increasing pressure to optimize feed utilization, commonly involving the use of cheap and more fibrous ingredients, there has been a concomitant increase in measures such as dietary enzyme addition to overcome problems resulting from the nature of the feeds themselves. Hindgut acidosis, a common condition associated with high levels of dietary carbohydrate (described in Review and interaction), is now recognised in ruminants, non-ruminant herbivores and humans and has recently been shown in pigs, dogs and mice. The range of symptoms and illnesses accrued through this acidosis are similar in all species and bear striking similarities to many problems in laying hens. This particularly applies to reductions in feed efficiency, disease conditions such as *E. coli*, coccidial and respiratory infections and, importantly for laying birds, mineral balance, skeletal abnormalities and plasma acid-base disturbances which impact upon the eggshell formation processes.

Socially, welfare considerations have a considerable impact on modern animal production as shown by increasingly stringent legislative regulation. The egg industry can only benefit by addressing potential welfare problems.

Environmental considerations are increasingly of concern to the egg industry largely due to pressure from urban encroachment into traditional intensive farming areas. This has resulted in the RIRDC Egg Program funding research into environmental, pollution and social conflicts. Projects have investigated "wet droppings" being influenced by diet and disease effects as well as reviewing water and particulate pollution that can by caused by egg production. It is suggested that these problems can be exacerbated by acidotic conditions in the bird's digestive process.

1.2 Relevance and benefits

The Australian egg industry has a gross value of production of $300M (AEIA, 1998). Eggshell downgrading results in the loss of some 7% of production. There had been no reduction in egg downgrading despite the best efforts of geneticists or nutritionists and it has been suggested (Solomon, 1990) that perhaps the wrong questions are being asked about dealing with the problem. Supporting this suggestion, Newman and Leeson (1997) argued that the answer to bone breakage in aged layers is not likely to be provided by nutritional manipulation. However, sub-optimal nutrition, even for short periods, may cause structural bone loss due to lack of medullary bone reserves (Whitehead and Wilson, 1992) and the loss is not replaced for the period that the bird is in lay thereby leading to degenerative bone problems. 15-30% of hen mortality in the U.S. has been ascribed to osteopenia (Roland and Rao, 1992).

The research outlined here is proposed to determine if laying birds suffer a fermentative or lactic acidosis as other animals do as no research has been directed towards this problem to date. It is possible that a hind gut acidosis, additive to an underlying metabolic acidosis such as occurs with egg shell formation, may contribute to some of the problems suggested above. An immune response, stimulated by such an acidosis, may underlie the reasons why nutritional manipulation appears to have little impact on improving eggshell losses or bone strength in layers.

Sustainability issues addressed by this project include improving welfare, immune status, feed utilisation, eggshell quality and skeletal integrity in the birds, reducing excreta moisture and nutrient losses and moderating the perceived negative influences of egg production (odour and water pollution) on neighbouring properties.

1.3 Review and interaction

1.3.1 Hindgut acidosis and laying hens

The range of effects of acidosis in ruminants, the horse and monogastric animals such as humans and pigs and the subsequent effects on the immune status of the animal, reviewed by Clayton (1999), mirror many conditions found in the laying bird. However, little research has been directed towards these effects of feeding increasingly cheap, fibrous materials to poultry. Perhaps the single most important indication of deleterious effects of nutrition upon immune status is provided by the increasing importance of enzyme technology in feeding. The mode of action of the major commercial enzymes is still not clearly understood and results are inconsistent (Bedford, 1997). It may be that means are being sought to counter the negative effects of feeding technology which is geared to the needs of automation (Summers and Leeson, 1979) and not the physiology of the bird.
Modern feeds are digested in a way that results in rapid throughput of the ration which is affected by particle size and moistening. The bird's metabolism is altered and the application of exogenous enzymes to the birds diet allows for the improved utilization of the diet via a reduction in digesta viscosity, which, in turn, is well correlated with feed conversion efficiency (Bedford, 1996). The addition of exogenous enzymes affects activities of endogenous enzymes suggesting that humoral regulation is important in the secretion of digestive enzymes (Han, 1996). pH is important for the activity of digestive enzymes (Marquardt and Bedford, 1996). For example, chicken α-amylase functions optimally between pH 7.0 and 8.0 (Gapusan *et al*., 1990). The influence of feed type and passage can have a profound influence on gut section pH and therefore α-amylase activity. The rate of α-amylase secretion is determined by the rate of carbohydrate metabolism (Rodeheaver and Wyatt, 1984) and this is further complicated by the differing amylase isozymes which have been found between strains of chickens (Yardley *et al*., 1988). Chicken amylase isozymes respond differently to wheat α-amylase inhibitors (Gapusan *et al*., 1990) and this may seriously influence results of carbohydrate digestibility trials. Hindgut acidosis may interact negatively with these processes.

1.3.2 Lactic Acidosis

Lactic acidosis is caused by excessive fermentation of carbohydrate leading to large quantities of lactic acid, and other organic acids, being produced in the gut (Clayton, 1999). Metabolic acidosis refers to a disturbance in blood acid-base balance and pH (Clayton, 1999). Lactic acidosis can cause metabolic acidosis in most animals and decreases in blood pH. Hughes (1988) detailed many of the consequences of acid-base disturbances in chickens.

Carbohydrate utilization in the hind-gut of ruminants
In ruminants, 18 to 40 % of starch from concentrates can reach the ileum (Owens *et al*., 1986) and this may result in a decrease in faecal pH from 6.9 to 6.0 indicating that starch has entered the caecum and led to metabolic changes (Russell *et al*., 1981). An increase in starch entering the caecum and fermenting may cause adverse effects such as an increased faecal N loss (Orskov, 1986) and diarrhoea (Mann and Orskov, 1973). Lactic acid producing gram positive bacteria such as *Streptococcus bovis* and *Lactobacillus sp*. increase in the caecum and pH may fall to below 5.0 (Allison *et al*., 1975) and caecal motility may be reduced (Dougherty *et al*., 1975). Scouring is an early indicator of acidosis (Ryan, 1964) as water reabsorption is reduced in the colon due to the acid load (Lee, 1977).

Monogastric animals and hind-gut acidosis
Fermentation patterns and increases in gram positive bacteria in the caecum of horses are similar to ruminants if horses are given an oat diet compared to a hay diet (Kern *et al.*, 1973). Caecal pH can fall after concentrate feeding to as low as 4.14 and caecal and plasma lactic acid concentrations rise which have been associated with lameness and laminitis (Garner *et al*., 1978) and neurological disturbances (Willard *et al*., 1977).
High dietary carbohydrate in pigs can cause greater caecal fermentation, produce a pH as low as 5.5 (Jensen and Jorgensen, 1994), and increase steam-volatile or short-chain fatty acid (VFA and SCFA respectively) levels and ileal lactic acid (Bach Knudsen and Hansen, 1991). Swine dysentery was related to caecal pH when carbohydrate reached the caecum intact and lowered pH and was exacerbated when corn and sorghum diets lowered the pH of the colon to 5.9 compared to boiled rice diets giving a pH of 6.5 (Pluske *et al.*, 1996).

Carbohydrate metabolism in the human gut can give similar bacterial, lactic acid and volatile fatty acid production as in ruminants (McNeill *et al.*, 1978; MacFarlane and Englyst, 1986). Dietary fibre in the caecum and colon increases fermentation and VFA levels (Mortensen *et al.*, 1988) with pH decreasing from 7.2 to 5.5 (McNeill *et al.*, 1978). Excess starch fermentation with wheat flour can lower pH. Carbohydrate malabsorption reduces pH (Flourie *et al*., 1986) and amino acid turnover (Mortensen, 1990) and causes high lactate levels (Holtug *et al*., 1992), cramping, flatulence and diarrhoea (Saunders and Wiggins, 1981). Cooked rice reduces bowel irritation and diarrhoea. Disease and surgical conditions may increase fermentation and can increase lactic acid and gram positive bacteria levels (Caldarini *et al*., 1996). Inflammatory Bowel disease (IBD) does, and coeliac disease may, cause metabolic acidosis from fermentation due to carbohydrate malabsorption. Acute lactic acidosis can affect neurological status, cause ataxia, lethargy (Caldarini *et al*., 1996), nausea and vomiting (Oh *et al*., 1979).

Physiological effects of acidosis

Acidosis damage to the gut wall can cause liver abscess incurred by micro-organisms entering the portal blood in lot fed cattle (Brent, 1976). Toxins have been implicated in hind gut acidosis responses. The caecal mucosa is damaged by acidosis which can lead to neurological abnormalitites associated with the acidosis in horses which may be mediated by endotoxin arising from the low caecal pH (Sprouse *et al*., 1987). Histamine and tyramine produce acidosis-like effects when given to healthy animals but lactic acid alone can induce epithelial damage (Ahrens, 1967). Cabrera-Saadoun and Sauveur (1987) found that histamine elicited bone resorption in laying hens during eggshell formation; a process causing a metabolic acidosis. The lipopolysaccharide (LPS) of the cell wall from gram-negative bacteria, killed by acidosis, is involved in acidosis induced diseases (Mullenax *et al.*, 1966). In commercial poultry operations, a major cause of mortality is due to secondary infection by gram-negative *E. coli*.

Mineral metabolism

Acid-base balance is extremely important in relation to eggshell quality (Hughes, 1988) and kidney function (Wideman, 1992) in layers. The kidney stabilises blood pH by adjusting the rate of H^+, fixed cation and anion excretion (Wideman, 1992). Acidosis inhibits carbonate formation and vitamin D activation and increases bone solubility and urinary calcium (Wideman, 1992). Blood pH is maintained in the range of 7.0-7.6 and large amounts of H^+ ions are produced by food and bone metabolism, shell formation and heat stress (Hughes, 1988). Eggshell formation involves severe metabolic acidosis (van de Velde *et al.*, 1986) which is exacerbated if large amounts of medullary bone must be mobilised and subsequently replenished due to dietary calcium shortage. Bone resorption is an acidic process (Miller, 1992). Acid-base balance is crucial in calcium metabolism, as active transport of calcium across the uterus requires a highly active form of $Mg^{2+}HCO3-ATPase$ which in turn requires carbonic anhydrase (Hughes, 1988).

Osteoclastic function is increased by metabolic acidosis causing bone resorption in avians (Corradino, 1973) and humans to release minerals to buffer the acid load (Bushinsky, 1995). The metabolic acidosis imposed in layers by eggshell formation may be partially buffered by the P fraction of the orthophosphate.

Skeletal and mineral metabolism, reviewed in detail by Loveridge *et al*. (1992), Thomson and Loveridge (1992) and Watkins (1992), is partly regulated through intermediaries including the cytokines 0but the influence of these factors may be greatly magnified in acidotic conditions. Metabolic acidosis restricts calcium absorption from the small intestine. A diet induced acidosis would have an additive effect on a metabolic acidosis.

There is no suggestion that an acute acidosis may occur due to cereal feeding in laying birds. Rather, a sub-acute or chronic underlying acidosis may be incurred with feeding high levels of individual cereals. This may be exacerbated, at times, when diet formulations are altered with subsequent use of different cereal types (based on price if for no other reason) and/or cereals sourced from different locations and, hence, potentially grown under widely varying conditions. In tandem with these considerations, the use of new season grains has been shown to be a particular problem at times. Furthermore, at certain times in the development of the bird, feed intake may increase rapidly i.e. post-point of lay and at peak production which may involve maladaptation of the gut to greater feed

volume in the short-term. This is certainly a consideration in broiler breeder systems where the laying birds, under long-term heavy feed restriction, are exposed suddenly to larger quantities of feed than they have been conditioned to eat. With egg laying, a greater "meal" feeding pattern may occur after the dark period with birds not eating until after oviposition. The consumption of a large "meal" may act in the same way as a novel cereal and result in carbohydrate overload with subsequent acidotic consequences.

Lactic acid in poultry and laying birds in particular has been studied largely in relation to metabolic acidosis or its production in the crop during fermentation of feed by micro-organisms. Bell and Culbert (1968) suggested that the origin of plasma lactate in hens was complex and indicated that mean levels of plasma lactate were much lower in mammals than in hens and that high egg production lines had greater levels than poorer producers. Perhaps importantly, Bell and Culbert (1968) concluded that either aerobically or anaerobically the birds' erythrocytes neither consumed glucose nor produced lactate but that activity was concentrated in the white cell population and in bone marrow cells. Frankel and Frascella (1968) provided supporting evidence by suggesting that increased plasma lactate due to an increase in tissue anaerobic glycolysis was moderated in chicken blood due to the aerobic metabolism of the nucleated erythrocytes of birds. Cortical bone could yield lactate as a product of glycolysis when bone demineralisation occurred daily (Bell and Culbert, 1968). It is reasonable to assume that this would apply equally to medullary bone which provides the more available and mobile calcium reserve for egg shell formation. In growers and layers, heat stress studies (Odom and Ono, 1991; Koelkebeck and Odom, 1994) indicated that the resultant acid-base disturbances highlighted a role for accumulation of plasma lactate as an extrarenal buffer to combat severe alkalosis. In studies of Fatty Liver and Kidney Syndrome and the association with lactate accumulation in blood, the conclusion of Balnave and Pearce (1979) that the lactate was a result of, rather than a contributory cause of FLKS, in broilers at least, also used the description lactic acidosis and is one of the few papers to do so. It is of note that Imaeda (2000) suggested that an increase in blood lactic acid damaged the cardiac system and could predispose broilers to sudden death syndrome.

The control of gut pathogens has been the focus of much study into dietary components, fermentation and lactic acid and VFA/SCFA production. In many studies there is concentration on one gut organ such as the crop (Corrier *et al*., 1999) or the caeca (Corrier *et al*., 1990). A comprehensive study of energy metabolisability and organic acid losses in excreta from broilers and adult cocks (Carre *et al*., 1995) focussed on dietary components of differing fermentability and quoted in the conclusions estimates of gross energy utilisation based on the hindgut fermentation efficiencies of pigs.

In most of the studies involving lactic acid measurement, total lactic acid or L-lactic acid was determined (it is often difficult to understand exactly what was measured) but the problems associated with D-lactic acid accumulation, as noted in a range of species from cattle, sheep and humans, has been little studied in the laying hen. Given the large component of the layer diet that is cereal grain, this project was designed to investigate the possibility that lactic acidosis may occur in the hindgut of layer-type birds when cereal changes were introduced at different stages in the life-cycle.

2. General Materials and Methods

2.1 Birds

Layer stock
Bartter Enterprises commercial tinted-egg layer cross (AZTEC x Lohmann Red) AZTEC 101 / 007. Fumigated eggs hatched at the Bartter Enterprises Beresfield commercial hatchery. Commercial vaccinations at day old in the hatchery and thereafter on farm.
Housing
Insulated, tunnel ventilated, concrete-floored shed. Electric brooders to 49 d thence floor rearing on litter. Trials conducted in Harrison carry-on cages.

2.2 Feeds

Commercial starter and grower diets
Millmaster starter crumbles with coccidiostat from day old to 8 weeks then grower crumbles were provided for rearing for experiments 1-5. Growers for the final two layer experiments were reared on feeds sourced from another miller. The experimental diets follow;

Table 1 Experimental grower diets (g/kg).

Raw	Wheat	Rice	Sorghum	Barley
Rice (80g/kg CP)		728.2		
Wheat (120 g/kg CP)	837.9			
Sorghum (90g/kg CP)			747.9	
Barley (100g/kg CP)				770.2
Soybean meal (475 g/kg CP)	25.0	90.0	143.0	70.0
Meat meal (520 g/kg CP)	70.0	90.0	12.0	68.0
Millrun (160 g/kg CP)	50.0	50.0	50.0	50.0
Sunflower oil	3.5	35.5	0.5	35.0
Limestone	5.0		19.5	
Lysine HCl	1.8	0.1	1.0	0.4
DL-Methionine	0.9	1.0	1.1	0.9
L-Threonine	0.4	0.2		
Salt	2.0	2.0	3.5	2.5
Dicalcium phosphate			18.0	
Choline chloride	1.0	0.5	1.0	0.5
Vitamin/mineral grower premix	2.5	2.5	2.5	2.5
Calculated specifications[1]				
DM	89.20	88.18	88.00	89.51
Protein	15.51	15.12	15.03	15.04
Fat EE	2.83	5.08	2.68	5.94
Linoleic	1.02	2.96	1.00	3.09
Ca	1.15	1.23	1.34	0.95
P	0.71	0.67	0.73	0.71
av. P	0.46	0.45	0.45	0.45
AME chick MJ/kg	11.91	11.91	11.91	11.92
Lysine	0.71	0.72	0.71	0.72
Methionine	0.31	0.35	0.32	0.31
Met + Cys	0.61	0.57	0.57	0.58
Threonine	0.51	0.52	0.53	0.52
Na	0.16	0.18	0.17	0.17
Cl	0.27	0.24	0.32	0.31

[1](% unless otherwise indicated)

Table 2 Experimental layer diets (g/kg).

Raw	Wheat	Rice	Sorghum	Barley
Rice (80g/kg CP)		600.0		

Wheat (120 g/kg CP)	673.3			
Sorghum (90g/kg CP)			600.0	
Barley (100g/kg CP)				600.0
Soybean meal (475 g/kg CP)	107.0	134.0	167.0	135.0
Meat meal (520 g/kg CP)	97.0	130.0	80.0	108.0
Millrun (160 g/kg CP)	30.0	49.1	55.1	32.2
Sunflower oil	11.3	12.5	8.5	10.0
Tallow		6.5		39.5
Limestone	74.0	61.5	81.0	69.0
Lysine HCl	0.8		0.4	
DL-Methionine	1.6	1.7	1.8	1.65
Salt	2.5	2.5	2.5	2.5
Dicalcium phosphate			1.2	
Choline chloride	0.5	0.2	0.5	0.15
Vitamin/mineral layer premix	2.0	2.0	2.0	2.0
Calculated specifications[1]				
DM	89.83	88.82	88.88	90.26
Protein	18.27	18.03	18.04	17.99
fat EE	3.63	3.88	3.83	7.51
Linoleic	1.41	1.42	1.41	1.43
Ca	4.04	4.00	4.09	4.00
P	0.84	0.90	0.78	0.90
av. P	0.56	0.63	0.45	0.61
AME chick MJ/kg	11.50	11.52	11.53	11.52
Lysine	0.88	0.90	0.88	0.91
Methionine	0.42	0.46	0.43	0.42
Met + Cys	0.74	0.71	0.70	0.72
Threonine	0.60	0.61	0.64	0.64
Na	0.21	0.24	0.19	0.21
Cl	0.30	0.29	0.29	0.32

[1](% unless otherwise indicated)

Table 3 Commercial laboratory analyses (duplicate samples) of experimental raws.

Raw	Protein (% as is) laboratory 1	Starch (% as is) laboratory 2	Starch (% dry matter) laboratory 2
Rice	8.1	74.0	64.5
Wheat	13.5	57.4	51.4
Sorghum	12.1	60.6	53.5
Barley	11.0	50.4	45.4
Soybean meal	47.4		
Meat meal	46.9		

2.3 Sample collections and measurements

In each experiment, birds of approximately equal body weight were transferred to individual trial cages and fed the commercial ration for 72 h prior to experimental treatments being imposed at lights

on on d 1 = 0 h. In many studies diets have been applied to birds for as little as 24 h (Balnave *et al*., 1977) or a maximum of 72 h (Carre *et al*., 1995) when measures of lactic acid and vfa's have been made.

One hour prior to each collection period, excreta trays were scraped clean. Fresh excreta (caecal content and urinary excretion contamination minimised) pH was measured after dilution 2-3 x deionised water (w/v) and a glass bead were added and mixed by vortex. pH was measured within 10 min of sample collection using a combination glass calomel pH probe (Selby Biolab). The methodology was similar to that used in ruminant studies (Clayton, 1999) and a similar type of study in broilers (Corrier *et al*., 1990). Samples were taken at 0 (feed change = control) and 12, 24 and 36 h. When labour permitted, another excreta measure was taken at 48 h. Similarly, extra collections were made in 12 hourly periods prior to the commencement of each experiment.

Fresh caecal evacuations were similarly measured when possible.

At 0 or 12 h thence 24, 36 and 48 h, blood samples of approximately 2 ml were collected into EDTA tubes and placed immediately on ice thence transferred to the laboratory for centrifugation for 10 min at 3,000 g. The plasma was transferred to a 2ml tube then frozen at –20° C. A sub-sample was retained for immediate determination of pH although in Experiments 1 and 2a the plasma for pH determination was frozen then thawed. Plasma pH was measured as above; based on a similar methodology applied by Frankel and Frascella (1968) who used a Metrohm micro-pH electrode.

The birds were euthanased at 48 h and gut section contents were collected in two separate tubes; one for determination of fresh digesta pH (treated as for excreta above), the other was placed on ice for transport to the laboratory. Kidneys were removed and placed in tubes on ice. The digesta pH (approximately 2 g in 4-6 g of deionised water) was measured within 20 min of death. Hinton *et al*., described pH measurement of 0.2 g of caecal digesta in 0.8 ml sterile and distilled water within 45 min of death. The kidneys were macerated in deionised water (added at 2 x kidney mass) and the pH measured as above.

Chilled digesta contents from the ileum, jejunum and crop (where collected) were centrifuged at 20,000 G for 20 min. Initially a 450 µL aliquot was placed into a glass GC vial with 50 µL of a 10 % formic acid/10% ortho-phosphoric acid plus 4-methyl valeric acid at 184 ppm standard solution. The tube was sealed, vortex mixed and frozen at –20 °C. Caecal contents had a 1% formic acid/1% ortho-phosphoric acid plus 4-methyl valeric acid (184 ppm) internal standard solution added 1:2 w/w, a glass bead was added and the contents vortex mixed until liquid then centrifuged as above. A 500 µL aliquot was added to a GC vial.

The final method employed for digesta preparation for VFA (SCFA) determination was based on the 1:2 w/w method for all samples. All vials were thawed and centrifuged for 5 min at 3,000 G prior to placement in the GC racks. The methodology was based on the SOP for VFA in rumen fluid by gas chromatography courteously provided by M. Davies, Agriculture Victoria Ellinbank Laboratory. Volatile fatty acid composition of samples was determined by capillary gas chromatography using 4-methyl-valeric acid as an internal standard. The method was developed anew in this laboratory employing an SGE BP21 analytical column, fitted with a retention gap kit and an SGE RGK2 guard column of 1m. An inlet volume of 1 µl at an inlet temperature of 155 °C and pressure of 8.2 kPa was used. The total flow was 48.7 ml min^{-1} and split ratio of 5:1. The carrier gas was ultra-pure H.

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 concentration (mmol/l) in the plasma and digesta supernatant was measured using a D-lactic acid/L-lactic acid test kit (Boehringer Mannheim) with a COBAS Bio (Roche

Diagnostics) centrifuge analyser. Samples were randomly tested for colour effects and spiked samples were employed to monitor the method.

2.4 Statistical analyses

Gut section digesta and kidney pH, individual volatile fatty acid concentration and VFA proportion of total VFA and lactic acid 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, excreta and caecal content pH and plasma pH were analysed using the PROC MIXED Model of SAS to accurately model the covariance structure of the repeated measures and to account for within-animal variance changes. Parameters expected to have possible pre- and post-treatment effects e.g. individual bird feed intake (d 1), excreta or plasma pH (time = 0) etc. were initially included in the model as a covariate. Where a relationship was not found (as occurred in most instances) the parameter was not included in the final analysis.

Relationships between various measures were tested using the GLM procedure of Minitab (Release 12.1).

2.5 Ethical considerations

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

3. Effects of a dietary cereal base change on gut conditions in female growers

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 assumed 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.

An initial experiment was required to determine effects of a change in the cereal base of a diet from a commercial diet fed long-term to young layer stock. Wheat, used by the commercial miller, of a known age and blend was selected for comparison with the commercial grower feed currently produced by the company. This was designed to develop methodology and to indicate if changes in fermentative activity occurred in various segments of the lower gut.

3.2 Materials and methods

As per General Materials and Methods (above). At 56 d old, the birds were allocated to treatments (n=18) at lights on on d 1 after 5 d in the experimental cages.

3.3 Results

Feed intake was similar ($p > 0.05$) on both the wheat mash (49.1 ± 1.17 g) diet and the commercial diet (47.7 ± 1.20 g) on d 1.

Excreta pH decreased ($p < 0.05$) when birds were fed the wheat feed (Table 3) but was unaltered ($p > 0.05$) by d 1 feed intake, excreta or plasma pH at time 0 i.e. on the commercial or control diet.

Caecal evacuations were little produced over the trial period which precluded useful pH testing.

Plasma pH was similar ($p > 0.05$) on the wheat diet and commercial diets at 48 h (8.218 and 8.233 ± 0.020 respectively).

Table 3. Influence of commercial or wheat-based diets on the fresh excreta pH of growers (n=18) at 8 weeks old.

Feed	Time (h)			
	0	12	24	36
Wheat	7.06	6.42 [b]	6.76 [b]	6.62 [b]
Commercial	7.27	7.28 [a]	7.36 [a]	7.46 [a]
SE		0.123		
Analysis		Covariate analysis		

Factor	p	Factor	p
Time	0.0880	Excreta pH Time 0	0.6833
Feed	**0.0001**	Feed intake d 1	0.4661
Time*Feed	**0.0296**	Plasma pH Time 0	0.9868

Plasma pH was not significantly altered ($p > 0.05$) over time on the wheat diet from 12, 24 36 and 48 h (8.306, 8.400, 8.369 and 8.218 respectively). A failure in blood collection procedure resulted in time 0 h collection for the wheat diet being discarded. Labour constraints prevented blood collections at 12, 24 and 36 h for the birds on the commercial diet.

Digesta content pH (Table 4) was higher ($p < 0.05$) in the gizzard and lower ($p < 0.05$) in the ileum and colon of birds fed the wheat diet compared with those maintained on the commercial diet. Digesta pH in the remaining gut sections and macerated kidney pH were similar ($p > 0.05$) across the feeds.

Table 4. Digesta and macerated kidney pH of growers maintained on a commercial grower ration or given a wheat-based feed for 48 h at 8 weeks old.

Gut section	Feed	n	LS Mean	Sd	Min	Max	SE (LS mean)	p
CROP	Wheat	17	4.58	0.261	4.2	5.2	0.072	0.116
	Commercial	18	4.74	0.327	4.3	5.5	0.070	
GIZZARD	Wheat	17	3.72 [b]	0.181	3.3	4.0	0.082	**0.019**
	Commercial	18	3.43 [a]	0.439	2.7	4.1	0.080	
DUODENUM	Wheat	17	6.58	0.205	6.1	6.9	0.042	0.140
	Commercial	18	6.49	0.132	6.1	6.7	0.040	
ILEUM	Wheat	17	8.47 [a]	0.532	7.2	9.4	0.108	**0.028**
	Commercial	18	8.82 [b]	0.342	8.0	9.2	0.105	
CAECUM	Wheat	17	5.48	0.693	4.6	7.1	0.144	0.426
	Commercial	18	5.64	0.484	5.1	7.1	0.140	
COLON	Wheat	17	8.43 [a]	0.787	6.6	9.2	0.138	**0.049**
	Commercial	18	8.82 [b]	0.213	8.5	9.3	0.134	
KIDNEY	Wheat	17	6.88	0.113	6.7	7.1	0.028	0.492
	Commercial	18	6.86	0.115	6.7	7.1	0.027	
PLASMA (control)	Wheat	17	8.22	0.147	8.0	8.5	0.038	0.768
	Commercial	18	8.23	0.164	8.0	8.5	0.037	

Blood collection failures (see plasma pH results above) only allowed for statistical analysis of results at 48 h and plasma L- or D-lactic acid (Table 5) were not significantly ($p > 0.05$) altered by feed type or method although at 48 h D-lactic acid levels were greater ($P < 0.05$) for both the diets than at time 0 on the commercial diet.

Table 5. Plasma concentration of L- and D-lactic acid (mMol/L) of growers (n=18) maintained on a commercial grower ration or given a wheat-based feed for 48 h at 8 weeks old.

Factor	Time (h)									
	0	12	24	36	48	0	12	24	36	48
	L-lactic acid					D-lactic acid				

Wheat		5.569	4.376	3.336	4.416		0.043	0.030	0.037	0.021
Commercial	3.952				4.442	0.007				0.020
SE					0.2531					0.0076
Analysis - main effects										
Factor			p					p		
Feed 48 h			0.9366					0.9556		

Digesta L- and D-lactic acid concentrations (Table 6) were not significantly altered ($p > 0.05$) by the feed in the ileum or caeca. L-lactic acid concentration across diets in the caeca approached significance ($p = 0.065$). The similar concentration of both isomers in the caeca should be noted particularly in contrast with relative levels in the ileum.

Table 6. Digesta L- and D-lactic acid concentration (mMol/L) in the distal ileum and caeca of growers maintained on a commercial grower ration or given a wheat-based feed for 48 h at 8 weeks old.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Ileum	Wheat	8.14	1.708	0.99	0.89	0.633	0.40
	Commercial	8.17	1.207		1.56	0.448	
Caecum	Wheat	8.19	1.496	0.065	7.15	1.488	0.15
	Commercial	4.14			4.06		

Jejunal, ileal (distal ileum) and caecal short chain fatty acid concentration (mMol/L) was similar ($P > 0.05$) across feeds other than for a reduction ($P < 0.05$) in heptanoic and total SCFA in the ileum after 48 h on the wheat-based feed (Table 7). The relative proportions (%) of the total SCFA (Table 8) were not significantly altered ($P > 0.05$) other than propionic acid being reduced in the caeca of birds fed the wheat-based diet for 48 h .

3.4 Discussion

Provision of a feed based solely on a high wheat inclusion produced a lower excreta pH which met the basic hypothesis that an acid response was caused with changes to the cereal base of a feed for layer-type birds. This simplistic interpretation of the excreta pH response was complicated by a time effect on the wheat feed whereby at 12 and 36 h pH was slightly lower than at 24 h. This was a numerical difference and raised the possibility that the effect of the feed moving through the digestive tract may culminate in considerable variation in digesta pH over the course of the day. Hill (1971) indicated ranges of digesta pH in the different organs but digestive/metabolic processes would be influenced by substrate availability over shorter time periods than one day.

Table 7. Influence of feeding a commercial grower diet or a wheat mash diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the mid-jejunum, distal ileum and caeca of 8 week old female growers.

Organ	Feed	N	Short chain fatty acid (C1-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Jejunum	Wheat	16	0.953	0.001	0	0.022	0.002	0	0	1.602	2.580
	Commercial	18	1.382	0	0	0.031	0	0	0	1.748	3.161
	SE Wheat		0.1886	0.0004		0.0100	0.0016			0.2097	0.3378
	SE Commercial		0.1778			0.0094	0.0015			0.1978	0.3185
	P		0.11	0.30		0.52	0.30			0.62	0.22
Ileum	Wheat	18	1.444	0.029	0.002	0.011	0.007	0	0.020	**0.631**	**2.143**
	Commercial	18	1.912	0.040	0.004	0.021	0.014	0	0.007	**0.940**	**2.938**
	SE		0.1747	0.0158	0.0017	0.0049	0.0042		0.0057	0.0981	0.2648
	P		0.07	0.61	0.36	0.16	0.27		0.12	**0.03**	**0.04**
Caeca	Wheat	17	34.501	1.418	5.011	0	0.263	0.023	0	0	41.216
	Commercial	18	37.612	1.827	3.994	0	0.290	0	0	0	43.723
	SE Wheat		2.8855	0.1945	0.7491		0.0614	0.0114			3.637
	SE Commercial		2.8042	0.1891	0.7280		0.0597				3.535
	P		0.44	0.14	0.34		0.75	0.15			0.62

Table 8. Influence of feeding a commercial grower diet or a wheat mash diet on the individual short chain fatty acid proportion (%) of total short chain fatty acid in the digesta content in the mid-jejunum, distal ileum and caeca of 8 week old female growers.

Organ	Feed	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Jejunum	Wheat	16	0.323	0.0002	0	0.009	0.0008	0	0	0.667
	Commercial	18	0.413	0	0	0.011	0	0	0	0.576
	SE Wheat		0.0396	0.00014		0.0037	0.00056			0.0415
	SE Commercial		0.0374			0.0035	0.00053			0.0392
	P		0.11	0.30		0.66	0.30			0.12
Ileum	Wheat	18	0.557	0.011	0.0005	0.004	0.002	0	0.007	0.251
	Commercial	18	0.658	0.017	0.0016	0.007	0.005	0	0.002	0.309
	SE		0.0448	0.0070	0.00066	0.0020	0.0015		0.0019	0.0246
	P		0.12	0.54	0.27	0.27	0.20		0.06	0.11
Caeca	Wheat	17	0.851	**0.034**	0.109	0	0.005	0.0007	0	0
	Commercial	18	0.863	**0.042**	0.089	0	0.006	0	0	0
	SE Wheat		0.0138	0.0029	0.0132		0.0011	0.00040		
	SE Commercial		0.0135	0.0028	0.0129		0.0010			
	P		0.56	**0.04**	0.29		0.51	0.20		

At 24 h the birds had only commenced to feed after a long dark period and were effectively meal feeding; the morning feed being quite substantial (intake not measured). Given that results for plasma lactate may have been similar at time 0, it is possible that lactate concentrations may have increased significantly between times 0 and 12 h, indicating an effect of an initial, single-cereal, feed. This highlighted a lapse in methodology which was to be corrected for subsequent trials. There was some question whether ethical standards may have been breeched by performing two bleeds from the same brachial vein with a haematoma. It was assumed that blood sampling at 0 h from the birds fed the commercial diet (effectively the control) should provide a baseline measure of plasma lactic acid as well as pH.

There was an indication that caecal lactic acid differences across the feeds correlated (non-significant) with ileal and caecal pH results. The ileal pH and VFA concentration was lower on the wheat diet and this lower pH could not be attributed to lactic acid. The numerically lower pH and VFA concentrations in the caeca in wheat-fed birds could be explained by lactic acid levels which were at least numerically higher with wheat feeding.

The experiment clarified improvements of methodology needed in subsequent work and a trial was devised to compare the wheat diet with a rice based diet (rice having minimal non-starch polysaccharide and a high starch content) for their effects on hindgut fermentation.

4. Effects of a wheat or rice-based diet on gut and digesta factors in female growers

4.1 Introduction

The different cereals have differing constituents and rice has almost no non-starch polysaccharide which provides the basis for problems of carbohydrate metabolism in the gut in cereals such as wheat. Although rice has not generally been a component of commercial diets it provides a good comparison for grain constituent effects as its starch is readily accessible for enzymatic activity in the gut. It is of note, however, that in the last season rice production was such that quantities of rice were cheap enough for inclusion in poultry diets in some areas. The effect of rice in the birds gut compared with wheat, when both grains provided single-cereal based diet exposure to birds previously fed a commercial blended diet, appeared to be the next logical step in determining effects on gut activity and function.

4.2 Materials and methods

As per General Materials and Methods (above). At 77 d old, the birds were allocated to treatments (n=18) at lights on on d 1 after 5 d in the experimental cages. An initial trial (Trial A) attempt failed as half of the birds on the rice diet failed to eat the mash which was largely an extremely fine mash. At 84 d-old, a new group of birds was trained to the equipment and feeds that had been cold-pelleted and crumbled (Trial B).

4.3 Results

Trial A.
Excreta pH was measured at feed change (control) and at 12 h and although pH dropped significantly ($p < 0.05$) from 7.17 ± 0.105 to 6.50 ± 0.101, irrespective of grain type ($p > 0.05$) the lack of feed intake in birds on the rice diet compromised the trial.

Trial B.
Feed intake was not influenced ($p > 0.05$) by grain type but increased ($p < 0.05$) from d1 (35.9 and 35.0 ± 2.31 g for wheat and rice respectively) to d 2 (50.1 and 50.6 ± 2.33 g for wheat and rice respectively).

Excreta pH increased ($p < 0.05$) at 36 h (Table 9) however there was no effect ($p > 0.05$) of grain type. Excreta pH from 12 to 36 h was not affected ($p > 0.05$) by excreta or plasma pH at time 0. Feed intake on d1 influenced subsequent excreta pH ($p < 0.05$). Few caecal evacuations were produced other than for the collection at 36 h and grain type did not alter ($p > 0.05$) pH (6.3 ± 0.21 and 6.7 ± 0.18 for wheat and rice respectively).

Plasma pH decreased ($p < 0.05$) over time (Table 10) and was reduced ($p < 0.05$) on the wheat diet (7.89 ± 0.010) compared with the rice diet (7.92 ± 0.010). Neither plasma nor excreta pH at time 0 influenced ($p > 0.05$) subsequent plasma pH. Comparison of plasma pH at 12 and 24 h in each trial confirmed the difference ($p < 0.05$) in pH between the wheat (7.887 ± 0.0095) and rice (7.923 ± 0.0100) diets.

Table 9. Influence of wheat or rice-based diets on excreta pH of growers (n=18) at 12 weeks old.

Feed	Time (h)			
	0	12	24	36
Mean pH	7.16 [b]	7.08 [b]	7.17 [b]	7.50 [a]
		0.104		
Wheat	7.20	7.01	7.25	7.68
Rice	7.12	7.14	7.09	7.32
SE		0.147		

Analysis		Covariate analysis	
Factor	p	Factor	p
Time	**0.0274**	Excreta pH Time 0	0.1732
Feed	0.2724	Feed intake d 1	**0.0024**
Time*Feed	0.4139	Plasma pH Time 0	0.3901

Table 10. Influence of wheat or rice-based diets on the frozen and thawed plasma pH of growers (n=18) at 12 weeks old.

Feed	Time (h)			
	12	24	36	48
Mean pH	8.223 [a]	7.901 [b]	7.830 [c]	7.667 [d]
SE	0.0139	0.0134	0.0144	0.0134

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	0.7080
Feed	**0.0109**	Feed intake d 1	Nil convergence
Time*Feed	0.7849	Plasma pH Time 0	0.3129

Grain type did not alter ($p > 0.05$) gut section digesta or macerated kidney pH (Table 11).

Table 11. Gut section digesta and kidney pH of birds euthanased after 48 h access to wheat or rice cold pelleted, crumbled feed.

Gut section	Feed	N	LS Mean	sd	min	max	SE (LS mean)	p
CROP	Wheat	18	4.96	0.624	4.40	6.20	0.113	0.945
	Rice	18	4.95	0.257	4.60	5.50	0.113	
GIZZARD	Wheat	18	3.88	0.707	2.80	5.30	0.130	0.610
	Rice	18	3.97	0.324	3.30	4.40	0.130	
DUODENUM	Wheat	18	6.48	0.211	6.10	6.90	0.050	0.187
	Rice	18	6.39	0.209	6.00	6.80	0.050	
ILEUM	Wheat	13	8.73	0.304	8.10	9.10	0.074	0.079
	Rice	8	8.96	0.192	8.70	9.20	0.095	
CAECUM	Wheat	18	6.10	0.491	5.40	7.10	0.100	0.095
	Rice	16	6.35	0.330	5.80	6.80	0.106	
COLON	Wheat	18	8.62	0.387	7.70	9.00	0.163	0.247
	Rice	18	8.35	0.899	6.20	9.20	0.163	
KIDNEY	Wheat	18	6.820	0.0770	6.67	7.00	0.0185	0.184
	Rice	18	6.790	0.0790	6.55	6.90	0.0185	

Plasma L- or D-lactic acid (Table 12) were not significantly ($p > 0.05$) (3.98 ± 0.156 and 0.031 ± 0.0053 and 4.43 ± 0.164 and 0.030 ± 0.0054 for L- and D-lactic acid from wheat and rice diets

respectively) although the L-isomer approached a significantly lower ($p = 0.051$) concentration on the wheat compared with the rice diet. The concentration of both isomers fell ($P < 0.05$) from 12 h to 24 h and increased ($p < 0.05$) to the original levels at 48 h in the case of L-lactic acid and at 36 h for the D- form.

Table 12. Effects of wheat or rice-based diets on the plasma concentration of L- and D-lactic acid (mMol/L) of growers (n=18) at 12 weeks old.

Feed	Time (h)							
	L-lactic acid				D-lactic acid			
	12	24	36	48	12	24	36	48
Mean concentration	4.87 [a]	3.61 [b]	3.22 [b]	5.13 [a]	0.039 [a]	0.008 [b]	0.048 [a]	0.028 [ab]
SE	0.221	0.221	0.241	0.221	0.0074	0.0074	0.0080	0.0074
Wheat	4.58	3.64	2.78	4.95	0.042	0.005	0.049	0.029
Rice	5.17	3.58	3.66	5.31	0.037	0.012	0.046	0.027
SE	0.313		0.368	0.313	0.0105		0.0119	0.0105

Analysis - main effects		
Factor	P	P
Time	**0.0001**	**0.0026**
Feed	0.0510	0.9209
Method	0.5228	0.9500

Digesta L- and D-lactic acid concentrations (Table 13) were not significantly altered ($p > 0.05$) by cereal type in the ileum or caeca. The relative concentrations of both isomers in each gut segment reflected those found in the initial experiment: D-lactic acid greatly reduced compared with the L-form in the ileum but with similar concentrations of each isomer in the caeca.

Table 13. Digesta L- and D-lactic acid concentration (mMol/L) in the crop, mid-jejunum, distal ileum and caeca of 12 week old growers after 48 h access to a wheat or rice-based diet.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Ileum	Wheat	4.79	0.726	0.69	1.38	0.541	0.36
	Rice	5.21			0.66		
Caecum	Wheat	0.72	0.164	0.86	0.78	0.134	0.17
	Rice	0.76	0.153		0.52	0.125	

Short chain fatty acid concentration (mMol/L) (Table 14) and proportion of total SCFA (Table 15) was similar for both feeds in all the gut sections other than for greater ($p < 0.05$) concentrations and proportions of heptanoic acid in the crop and propionic and *iso*-valeric acids in the caeca of birds given the rice-based diet.

Table 14. Influence of feeding a cold-pelleted and crumbled wheat or rice-based diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the crop, mid-jejunum, distal ileum and caeca of 12 week old female growers.

Organ	Feed	N	Short chain fatty acid (C1-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Crop	Wheat	15	5.279	0	0	0	0	0	0	**0.053**	5.332
	Rice	14	6.545	0.007	0	0	0	0	0	**0.156**	6.714
	SE Wheat		0.5425							0.0339	0.5430
	SE Rice		0.5615	0.0050						0.0351	0.5621
	P		0.12	0.31						**0.04**	0.09
Jejunum	Wheat	17	0.839	0	0	0.004	0	0	0	0.193	1.035
	Rice	18	0.551	0	0	0.004	0	0	0	0.151	0.707
	SE Wheat		0.1714			0.0026				0.0945	0.2535
	SE Rice		0.1666			0.0025				0.0918	0.2464
	P		0.24			0.94				0.75	0.36
Ileum	Wheat	17	1.629	0	0	0.002	0	0	0	0.126	1.758
	Rice	16	0.885	0	0	0.001	0	0	0	0.121	1.007
	SE Wheat		0.3189			0.0017				0.0702	0.3611
	SE Rice		0.3287			0.0018				0.0724	0.3722
	P		0.11			0.67				0.96	0.16
Caeca	Wheat	18	73.675	**3.065**	10.477	0.014	0.824	**0.020**	0	0	88.076
	Rice	18	60.922	**4.772**	7.243	0.107	1.004	**0.137**	0	0	74.184
	SE		5.1692	0.4616	1.6058	0.0355	0.1592	0.1592			6.4347
	P		0.09	**0.01**	0.16	0.07	0.43	**0.02**			0.14

Table 15. Influence of feeding a cold-pelleted, crumbled wheat or rice-based diet on the individual short chain fatty acid proportion (%) of total short chain fatty acid in the digesta content in the crop, mid-jejunum, distal ileum and caeca of 12 week old female growers.

Organ	Feed	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Crop	Wheat	15	0.988	0	0	0	0	0	0	**0.012**
	Rice	14	0.973	0.001	0	0	0	0	0	**0.025**
	SE Wheat		0.0057	0.0007						0.0050
	SE Rice		0.0059	0.0008						0.0052
	P		0.10	0.31						**0.04 ***
Jejunum	Wheat	17	0.910	0	0	0.010	0	0	0	0.080
	Rice	18	0.873	0	0	0.009	0	0	0	0.118
	SE Wheat		0.0388			0.0046				0.0400
	SE Rice		0.0378			0.0045				0.0389
	P		0.50			0.93				0.51
Ileum	Wheat	17	0.970	0	0	0.002	0	0	0	0.028
	Rice	16	0.905	0	0	0.001	0	0	0	0.094
	SE Wheat		0.0313			0.0015				0.0314
	SE Rice		0.0323			0.0016				0.0324
	p		0.16			0.71				0.15
Caeca	Wheat	17	0.835	**0.039**	0.115	0.0002	0.010	**0.0003**	0	0
	Rice	18	0.835	**0.066**	0.083	0.0014	0.012	**0.0022**	0	0
	SE		0.0145	0.0047	0.0137	0.00045	0.0016	0.00054		
	P		0.99	**0.001**	0.10	0.08	0.23	**0.02**		

* significant P of transformed data

4.4 Discussion

The use of wheat or rice based diets produced similar results in most measures in digesta, excreta and plasma. Reductions in plasma pH were consistent over the 48 h of the experiment and this requires elucidation as a continual reduction in plasma buffering capacity after application of a single-cereal based diet could have negative effects on eggshell formation.

Excreta pH was alkaline but with some perturbations evident at each time. The numerical, albeit non-significant, decline at 12 h may support the previous trial result with the daily feed load having produced an effective pH reduction at the end of the day and the unfed bird excreting a slightly more alkaline pH at 24 h following the utilisation of most feed constituents through the birds' or microbial processes. However, the higher pH at 36 h is somewhat contradictory to this suggestion. Generally throughout the digestive tract pH was within the limits defined by Hill (1971) other than ileal digesta which, irrespective of the cereal used, was almost one unit higher. The reason for this slightly higher alkalinity is unknown.

VFA concentrations, particularly total and acetic, in the ileum and caeca, were numerically higher in the birds fed the wheat diet. The rice diet produced a significantly greater concentration of propionic and iso-valeric acids in the caeca. Together with the first experiment, the molar ratios of acetic:propionic:butyric were considered unusual with a predominance of acetic acid being produced, unlike the values suggested from the work of Corrier *et al*. (1990) and Williams *et al*. (1997).

Lactic acid levels were similar with both the wheat and rice diets. The concentrations were considered to be of little concern although any measurable concentration of D-lactic acid may indicate the potential for problems associated with acidosis; a point emphasised in the results of Omole *et al*. (2001) who indicated no measurable D-lactate in healthy calves compared with diarrheic calves with metabolic acidosis.

In this experiment, little evidence was found that suggested that single-cereal based diets, either a wheat or rice diet, produced an acidotic effect in young growers. A range of commercially important cereals with differing constituents were of interest in their effects in the hindgut.

5. Comparison of wheat, rice, sorghum and barley-based diets on gut and digesta factors in female growers

5.1 Introduction

The cereals of commercial interest are structurally and chemically different. The effect that these differences have on grain and thus bird performance can be indirectly considered by the range of feed additives, particularly the endogenous feed enzymes, available for inclusion in poultry diets.

Recently, diets for laying birds have received considerable attention from commercial additive manufacturers and a range of feed enzymes have been produced to target most of the important cereals used for egg producing birds in Australia.

The digestive system of the bird takes some time to develop and attain its full adult capacity. At four months of age digestive physiology is considered to be mature. The following experiment was undertaken to investigate the effects of a range of commercial cereals in growers old enough to have such a mature digestive system. Of particular interest was a barley-based diet, given the practices of a layer producer in the Tamworth area of N.S.W. who formulated and milled full barley diets for growers and layers and had, after several years trial, ceased inclusion of any exogenous enzymes in such diets.

Cereals were old-season grain sourced from a commercial feed miller; cereals then currently in use in diets, albeit generally blended, for both growers and layers.

5.2 Materials and methods

As per General Materials and Methods (above). At 91d old, the birds were allocated to treatments (n=12) at lights on on d 1 after 5 d in the experimental cages. The method of plasma pH measurement was altered from that specified in the General Materials and Methods. Blood was placed on ice and transported to the laboratory for centrifugation and immediate plasma pH measurement. The process was repeated after each sampling and was completed within 3 hr. The plasma samples were then frozen at -20°C and measured after thawing to room temperature as per the previous experiments. A comparison of pH was then made for the two methods.

5.3 Results

Feed intake (g) was not influenced ($p > 0.05$) by grain type (47.5, 47.2, 48.9 and 45.5 ± 3.40 g for wheat, rice, sorghum and barley respectively) but increased ($p < 0.05$) from d1 to d2 (41.9 and 52.6 ± 2.40 g).

Excreta pH (Table 16) altered over time ($p < 0.05$) with excreta pH lower at 24 h than at 0, 12 or 36 h and sorghum produced a higher excreta pH ($p < 0.05$) than the other cereals (7.58^{b} ± 0.077, 7.47^{b} ± 0.077, 8.06^{a} ± 0.075 and 7.58^{b} ± 0.076 for wheat, rice, sorghum and barley respectively). Excreta pH from 12 to 36 h was not affected ($p > 0.05$) by excreta or plasma pH at time 0. Feed intake on either d 1 or d 2 similarly had no influence on excreta pH ($p < 0.05$).

Table 16. Influence of wheat, rice, sorghum or barley-based diets on the fresh excreta pH of growers (n=12) at 13 weeks old.

Factor	Time (h)			
	0	12	24	36
Mean pH	7.65 [a]	7.82 [a]	7.43 [b]	7.78 [a]
SE	0.075	0.076	0.077	0.077
Wheat	7.65	7.61	7.31	7.75
Rice	7.61	7.49	7.30	7.52
Sorghum	7.88	8.37	7.99	8.03
Barley	7.47	7.84	7.16	7.85
SE		0.164		

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0020**	Excreta pH Time 0	0.4552
Feed	**0.0001**	Feed intake d 1 and d 2	Nil convergence
Time*Feed	0.2822	Plasma pH Time 0	0.8036

Few caecal evacuations could be collected other than for 24 samples at 12 h and grain type did not alter ($p > 0.05$) pH (7.92, 6.94 and 7.18 ± 0.209 and 6.74 ± 0.155 for wheat, rice, sorghum and barley respectively).

Plasma pH (Table 17) decreased ($p < 0.05$) from 12 to 24 h, increased ($p < 0.05$) from 24 to 36 h then decreased ($p < 0.05$) substantially again from 36 to 48 h. Plasma pH was reduced ($p < 0.05$) on the wheat diet compared with the rice, sorghum and barley diets (7.692 [b] ± 0.0075, 7.729 [a] ± 0.0083, 7.730 [a] ± 0.0076 and 7.722 [a] ± 0.0078, respectively). Excreta pH at time 0 did not influence ($p > 0.05$) subsequent plasma pH. The initial plasma pH influenced ($p < 0.05$) subsequent plasma pH as did feed intake on d 1 ($p < 0.05$) but did not alter the significance of the results of the different factors.

Table 17. Influence of wheat, rice, sorghum or barley-based diets on the chilled plasma pH of growers (n=12) at 13 weeks old.

Factor	Time (h)			
	12	24	36	48
Mean pH	7.745 [a]	7.723 [b]	7.747 [a]	7.658 [c]
SE	0.0075	0.0077	0.0085	0.0075
Wheat	7.742	7.687	7.719	7.623
Rice	7.752	7.725	7.760	7.681
Sorghum	7.760	7.743	7.762	7.658
Barley	7.729	7.741	7.749	7.673
SE		0.0199		

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	0.4552
Feed	**0.0015**	Feed intake d 1	**0.0002**
Time*Feed	0.5360	Plasma pH Time 0	**0.0093**

Chilled plasma pH was increased ($p < 0.05$) with freezing (7.745 versus 8.298 ± 0.0130); a process not altered ($p > 0.05$) by the cereal grain fed.

Grain type did not alter ($p > 0.05$) gut section digesta or macerated kidney pH (Table 18) other than barley producing a lower ($p < 0.05$) crop pH than rice with the wheat and sorghum intermediate.

Table 18. Gut section digesta and kidney pH of birds euthanased after 48 h access to wheat, rice, sorghum or barley-based cold pelleted, crumbled feed.

Gut section	Treatment	N	LS Mean	sd	min	max	SE (LS mean)	P
CROP	Wheat	12	5.15 [ab]	0.707	4.3	6.2	0.177	**0.040**
	Rice	11	5.46 [b]	0.626	4.6	6.4	0.185	
	Sorghum	11	5.00 [ab]	0.635	4.5	6.1	0.185	
	Barley	12	4.70 [a]	0.454	4.0	5.4	0.177	
GIZZARD	Wheat	12	3.95	0.526	2.7	4.9	0.141	0.479
	Rice	12	3.85	0.342	3.2	4.4	0.141	
	Sorghum	12	3.80	0.649	2.0	4.6	0.141	
	Barley	12	3.64	0.377	2.9	4.2	0.141	
DUODENUM	Wheat	12	6.46	0.218	6.1	6.8	0.064	0.472
	Rice	12	6.32	0.325	5.4	6.7	0.064	
	Sorghum	12	6.42	0.205	6.0	6.7	0.064	
	Barley	12	6.40	0.06	6.3	6.5	0.064	
ILEUM	Wheat	7	8.94	0.222	8.7	9.3	0.098	0.489
	Rice	4	8.75	0.264	8.4	9.0	0.129	
	Sorghum	9	8.80	0.282	8.3	9.2	0.086	
	Barley	8	8.75	0.256	8.3	9.1	0.091	
CAECUM	Wheat	11	6.07	0.749	5.5	8.2	0.192	0.056
	Rice	12	6.72	0.549	5.9	7.4	0.184	
	Sorghum	12	6.26	0.536	5.3	7.1	0.184	
	Barley	12	6.08	0.692	5.4	7.6	0.184	
COLON	Wheat	12	8.37	0.825	6.3	9.0	0.159	0.875
	Rice	9	8.43	0.55	7.5	9.0	0.184	
	Sorghum	10	8.55	0.356	7.9	9.0	0.175	
	Barley	12	8.51	0.275	7.9	8.8	0.159	
KIDNEY	Wheat	12	6.760	0.1040	6.60	6.94	0.0289	0.770
	Rice	12	6.790	0.1190	6.54	7.04	0.0289	
	Sorghum	12	6.780	0.1050	6.56	6.91	0.0289	
	Barley	12	6.800	0.0590	6.69	6.89	0.0289	

Plasma L- or D-lactic acid concentrations (Table 19) were not significantly ($p > 0.05$) altered by feed type. L-lactic acid concentration fell ($p < 0.05$) from 12 to 24 h then lower ($p < 0.05$) to 36 h before a return to the original level at 48 h. D-lactic acid levels displayed a different pattern with a reduction ($p < 0.05$) from 12 to 24 h, a return to the original level ($p > 0.05$) at 36 h and a reduction ($p < 0.05$) again at 48 h with the 24 and 48 h levels being similarly ($P > 0.05$) low. The pattern of concentration changes was similar across all four grain types over the 12 to 48 h period.

Table 19. Effects of wheat, rice, sorghum or barley-based diets on the plasma concentration of L- and D-lactic acid (mMol/L) of growers (n=12) at 13 weeks old.

Factor	Time (h)							
	12	24	36	48	12	24	36	48
	L-lactic acid				D-lactic acid			
Plasma concentration (mMol/L)	4.97 [a]	4.17 [b]	3.51 [c]	4.70 [a]	0.079 [a]	0.009 [b]	0.091 [a]	0.031 [b]
SE	0.164		0.167	0.175	0.0116	0.0114	0.0226	
Wheat	4.62	4.07	3.47	5.09	0.073	0.003	0.083	0.022
Rice	4.95	4.07	3.46	4.34	0.043	0.012	0.089	0.012
Sorghum	5.04	4.46	3.57	5.03	0.074	0.017	0.103	0.058
Barley	5.29	4.09	3.56	4.33	0.125	0.004	0.088	0.033
SE	0.327		0.342	0.359	0.0226		0.0237	0.0226

Analysis - main effects		
Factor	P	P
Time	**0.0001**	**0.0001**
Feed	0.5959	0.3216
Time*Feed	0.7654	0.7179

Digesta L- and D-lactic acid concentrations in the ileum and caecum (Table 20) were not significantly altered ($p > 0.05$) by cereal type.

Table 20. Digesta L- and D-lactic acid concentration (mMol/L) in the distal ileum and caeca of 13 week old growers (n=12) after 48 h access to wheat, rice, sorghum or barley-based diets.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	P
Ileum	Wheat	5.85	1.215	0.92	1.41	0.765	0.43
	Rice	6.12	1.158		1.12	0.729	
	Sorghum	6.70	1.158		2.65	0.729	
	Barley	5.60	1.158		1.21	0.729	
Caecum	Wheat	1.85	0.720	0.24	1.62	0.700	0.24
	Rice	0.86	0.755		0.61	0.734	
	Sorghum	1.63	0.720		1.32	0.700	
	Barley	2.97	0.689		2.64	0.700	

Plasma pH and plasma total lactic acid were related ($R^2 = 0.70$) with lower total plasma lactic acid and high plasma pH produced by the rice and barley diets while wheat produced greater lactate and a lower pH. A log relationship between caecal pH and total lactate was apparent ($R^2 = 0.86$). Caecal pH was similarly well related to caecal VFA concentration ($R^2 = 0.81$) and total organic acids (R2 = 0.904).

Crop, jejunal, ileal (distal ileum) and caecal short chain fatty acid concentration (mMol/L) (Table 21) was similar ($P > 0.05$) across feeds other than for the presence of ($P < 0.05$) propionic acid in the ileum of birds on the wheat-based feed and n-butyric acid concentrations in the caeca being greater ($P < 0.05$) in birds fed the barley-based diet than those fed the rice or sorghum-based feeds with wheat intermediate. Total SCFA in the caeca of birds on the barley-based feed were greater ($P < 0.05$) than the rice-fed birds with wheat and sorghum intermediate. The relative proportions (%) of the total SCFA (Table 22) were not significantly altered ($P > 0.05$) other than for the presence of propionic acid in the ileum of birds fed the wheat-based diet for 48 h.

Table 21. Influence of feeding a cold-pelleted and crumbled wheat, rice, sorghum or barley-based diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the crop, mid-jejunum, distal ileum and caeca of 13 week old female growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	Hexanoic	heptanoic	Total
Crop	Wheat	7	5.185	0	0	0.002	0.057	0	0	0.057	5.301
	Rice	7	6.028	0	0	0.030	0	0	0	0.039	6.096
	Sorghum	11	8.714	0.036	0.014	0.036	0.056	0	0.008	0.163	9.028
	Barley	12	6.568	0	0	0.022	0.088	0	0	0.085	6.763
	SE W R		1.2462			0.0143	0.0491			0.0566	1.2417
	SE Sorghum		0.9941	0.0105	0.0046	0.0114	0.0392		0.0032	0.0452	0.9905
	SE Barley		0.9518			0.0109	0.0375			0.0432	0.9483
	P		0.14	0.06	0.10	0.30	0.57		0.20	0.31	0.10
Jejunum	Wheat	12	0.147	0.0163	0.0108	0.055	0.0197	0	0.009	0.378	0.636
	Rice	11	0.097	0.0002	0.0004	0.075	0.0046	0	0	0.418	0.596
	Sorghum	11	0.120	0	0	0.116	0	0	0	0.218	0.454
	Barley	12	0.159	0	0	0.111	0	0	0	0.173	0.443
	SE W B		0.0689	0.00589	0.00376	0.0295	0.00719		0.0034	0.0919	0.1564
	SE R S		0.0720	0.00616	0.00393	0.0308	0.00751			0.0959	0.1634
	P		0.93	0.15	0.13	0.42	0.19		0.13	0.20	0.77
Ileum	Wheat	12	1.591	**0.021**	0.0090	0.018	0.018	0.00003	0.006	0.454	2.116
	Rice	12	0.923	**0**	0	0.015	0	0	0	0.350	1.288
	Sorghum	12	0.999	**0**	0	0.012	0	0	0	0.258	1.269
	Barley	11	0.973	**0**	0	0.011	0	0	0	0.325	1.309
	SE W R S		0.1914	0.0062	0.00300	0.0058	0.0060	0.000017	0.0025	0.0746	0.2526
	SE Barley		0.1999			0.0061				0.0779	0.2638
	P		0.058	**0.046**	0.10	0.83	0.11	0.42	0.31	0.33	0.059
Caeca	Wheat	12	64.075	3.126	**7.983** [ab]	0.060	0.670	0.081	0.028	0.015	**76.036** [ab]
	Rice	12	48.618	3.942	**4.545** [a]	0.051	0.593	0.115	0	0	**57.864** [a]
	Sorghum	12	61.592	3.806	**7.248** [a]	0	0.459	0	0	0	**73.106** [ab]
	Barley	12	74.479	4.262	**12.667** [b]	0.038	0.983	0	0	0	**92.431** [b]
	SE		6.5031	0.4209	1.6775	0.0436	0.1427	0.0496	0.0138	0.0075	7.8743
	P		0.059	0.29	**0.01**	0.78	0.08	0.26	0.40	0.40	**0.03**

Table 22. Effects of cold-pelleted, crumbled wheat, rice, sorghum or barley-based diets on individual SCFA proportion (%) of total SCFA in the digesta in the crop, mid-jejunum, distal ileum and caeca of 13 week old female growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Crop	Wheat		0.971	0	0	0.0004	0.006	0	0	0.022
	Rice		0.989	0	0	0.0050	0	0	0	0.006
	Sorghum		0.953	0.005	0.0017	0.0035	0.005	0	0.0009	0.031
	Barley		0.963	0	0	0.0033	0.012	0	0	0.022
	SE W R		0.0177			0.00181	0.0058			0.0177
	SE Sorghum		0.0141	0.0017	0.00048	0.00145	0.0046		0.00032	0.0141
	SE Barley		0.0135			0.00138	0.0044			0.0135
	P		0.46	0.11	0.0501	0.35	0.43		0.19	0.75
Jejunum	Wheat	12	0.170	0.0119	0.0080	0.249	0.0147	0	0.007	0.457
	Rice	11	0.104	0.0002	0.0005	0.363	0.0060	0	0	0.526
	Sorghum	11	0.141	0	0	0.443	0	0	0	0.416
	Barley	12	0.171	0	0	0.360	0	0	0	0.386
	SE W B		0.0602	0.00419	0.00278	0.1033	0.00578		0.0025	0.1016
	SE R S		0.0629	0.00438	0.00290	0.1079	0.00603			0.1061
	P		0.85	0.13	0.13	0.63	0.25		0.12	0.80
Ileum	Wheat	12	0.744	**0.007**	0.003	0.012	0.006	0.000008	0.002	0.225
	Rice	12	0.710	**0**	0	0.013	0	0	0	0.277
	Sorghum	12	0.800	**0**	0	0.013	0	0	0	0.188
	Barley	11	0.758	**0**	0	0.011	0	0	0	0.231
	SE W R S		0.0324	0.0020	0.0010	0.0055	0.0022	0.0000042	0.0010	0.0351
	SE Barley		0.0338			0.0057				0.0367
	P		0.28	**0.04**	0.10	0.99	0.13	0.42	0.33	0.37
Caeca	Wheat	12	0.845	0.049	0.093	0.0018	0.008	0.002	0.0008	0.0004
	Rice	12	0.848	0.072	0.068	0.0010	0.009	0.002	0	0
	Sorghum	12	0.846	0.057	0.091	0	0.006	0	0	0
	Barley	12	0.810	0.049	0.131	0.0003	0.010	0	0	0
	SE		0.0149	0.0066	0.0156	0.00103	0.0016	0.0011	0.00040	0.00022
	P		0.24	0.057	0.053	0.63	0.34	0.29	0.40	0.40

5.4 Discussion

Contrary to the earlier trials the plasma pH did not decline consistently over the trial period with a higher pH registered at 36 h than at 24 h. No explanation is offered for this change although the lower overall pH produced by the wheat diet may not have accrued in the same way to responses to the other three cereals. The birds fed the wheat diet had numerically lower plasma pH at 24 and 36 h. Of note were the, at times, seemingly contradictory results for excreta pH compared with plasma pH. This is worthy of further detailed investigation.

It is unfortunate that the 0 h plasma pH and lactic acid was not measured as the differences in the concentrations of lactic acid isomers over succeeding time periods diverged according to isomer. The D-form displayed a saw-tooth pattern which appeared to match the time of feeding. The birds, although given free access to the feed from lights-on on day 1 (time 0), are in some way meal-feeding with consumption of a large meal thence small bouts throughout the day. The D-lactic acid levels were highest at 12 h after this morning feed i.e. at 12 and 36 h and lower at the time of bleeding within a short period of access to the morning feed. Therefore, the delay with digesta passage leads to a diurnal pattern of blood plasma lactic acid content and the accumulation of D-lactic acid displays this pattern clearly.

In the ileum there were no significant relationships between digesta pH and the lactate or VFA concentrations other than for the presence of a small concentration of propionic acid in birds given the wheat-based diet. In the caeca, calculation of the relationships between lactate and VFA revealed a pattern with the rice diet producing a higher pH and lower lactate concentration. The pH was higher on the rice diet than with sorghum, barley or wheat and the total organic acid concentration was lower than from the barley diet. Again, more replication may have allowed for considerably more detail to have been gleaned from these relationships. Nevertheless, of note for future work is the observation that barley produced high lactate and VFA concentrations associated with a low pH and rice produced a low lactate concentration with an associated high pH. Wheat and sorghum produced results between the above two cereals.

Although ileal digesta pH was both very high and similar or consistent across all four cereals, the greater differences in caecal digesta pH across the four cereals approached significance. A pH of any specific reading may not in itself indicate a potential problem due to diet. One experiment that would be valuable to perform in future work would be sequential measurement of digesta pH along the tract over short periods of time post-feeding. The relatively low pH found in the caecal digesta of birds fed the wheat and barley diets compared with the sorghum and especially the rice diets may not be problematic if it occurred over a prolonged period. Rather it is a sudden reduction in pH that would be of concern and a pH change of a little as half a unit may result in the onset of disease symptoms as noted in the results of Clayton (1999) and Clayton and Buffinton (2000).

6. Comparison of wheat, rice, sorghum and barley-based whole-grain diets on gut and digesta factors in growers

6.1 Introduction

The previous experiment produced results that suggested some manipulation of the cereals may be of interest. The grain processing alterations completed in the additional broiler trials (see results below) were performed on different cereals in separate experiments.

Considerable recent research has been devoted to the addition of whole grain (generally wheat) to pelleted rations for broilers. This work has been almost exclusively performed in Europe and production and mortality advantages have been consistent. For other reasons such as litter maintenance, local integrators have used the practice in breeder flocks and at times, litter-grown layers have been provided with whole grain. Although data is hard to access, anecdotal reports suggest, again, some production advantages.

Another motivation for whole grain inclusion in pelleted diets was the seemingly standard practice of performing AME (Apparent Metabolisable Energy) trials with whole grain put through a pellet press. Changing the form of the major component in tests of additives would appear to add a complicating factor when the birds, whether broiler, breeder or layer-types, have been grown on fully ground or ground and pelleted/crumbled diets.

The following trial compared the four cereals with the grain included, whole, in the mix prior to cold-pelleting.

6.2 Materials and methods

As per General Materials and Methods (above). At 98 d old, the birds were allocated to treatments (n=12) at lights on on d 1 after 5 d in the experimental cages. Feeds were as in the previous experiment with the cereal component of each diet included in the mix as whole grains. The feed was cold-pelleted and crumbled. Blood collections were limited to three due to welfare/research ethics considerations of taking a second sample from a brachial vein with an intact haematoma. The collections were at 0, 24 and 48 h; approximately 1h post-feeding at lights-on.

6.3 Results

Feed intake (g) was not influenced ($p > 0.05$) by grain type (74.5, 71.6, 74.6 and 73.1 ± 1.87 g for wheat, rice, sorghum and barley respectively) but decreased ($p < 0.05$) from d1 to d2 (76.1 and 70.8 ± 1.28 g).

Excreta pH (Table 23) altered over time ($p < 0.05$) with excreta pH lower ($p < 0.05$) at 12 and 36 h after feeding than the initial pH (measured prior to feeding at –12 and 0 h) but increasing ($p < 0.05$) at 48 h. Wheat and sorghum produced a higher ($p < 0.05$) pH than rice and barley (7.62 and 7.72 versus 7.43 and 7.37 ± 0.060). Excreta pH was not affected ($p > 0.05$) by excreta pH at time 0 or the quantity of feed intake.

Table 23. Influence of wheat, rice, sorghum or barley-based whole-grain diets on the fresh excreta pH of growers (n=12) at 14 weeks old.

Factor	Time (h)				
	-12	0	12	36	48
Mean pH	7.67 [b]	7.73 [b]	7.10 [a]	7.13 [a]	8.05 [c]
SE			0.067		
Wheat	7.63	7.90	7.28	7.22	8.13
Rice	7.41	7.52	7.22	7.12	7.90
Sorghum	7.81	8.00	7.17	7.30	8.35
Barley	7.84	7.54	6.78	6.89	7.83
SE			0.134		

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	0.3162
Feed	**0.0001**	Feed intake d 1	0.6832
Time*Feed	0.1488		

Few caecal evacuations could be collected and caecal content pH, measured at 0 and 12 h, did not differ ($p > 0.05$) over time (7.16 ± 0.100 and 7.32 ± 0.070) or with cereal type (7.35 ± 0.108, 7.06 ± 0.145, 7.30 ± 0.108 and 7.26 ± 0.122 for wheat, rice, sorghum and barley respectively).

Plasma pH (Table 24) decreased ($p < 0.05$) from 0 to 24 h then increased ($p < 0.05$) to the original value at 48 h. Plasma pH was similar ($P > 0.05$) on the wheat, rice and sorghum diets and lower ($P < 0.05$) than on the barley diet (7.653 [b] ± 0.0071, 7.656 [b] ± 0.0073, 7.664 [b] ± 0.0072 and 7.693 [a] ± 0.0071. Excreta and initial plasma pH at time 0 did not influence ($p > 0.05$) subsequent plasma pH.

Table 24. Influence of wheat, rice, sorghum or barley-based whole-grain diets on the chilled plasma pH of growers (n=12) at 14 weeks old.

Factor	Time (h)		
	0	24	48
Mean pH	7.676 [a]	7.641 [b]	7.681 [a]
SE	0.0062	0.0064	0.0062
Wheat	7.677	7.615	7.667
Rice	7.664	7.629	7.673
Sorghum	7.664	7.654	7.673
Barley	7.699	7.668	7.713
SE		0.0135	

Analysis	
Factor	P
Time	**0.0001**
Feed	**0.0003**
Time*Feed	0.5728

After 48 h on-feed, gut section digesta and macerated kidney pH (Table 25) revealed differences ($p < 0.05$) across grain type in the crop, gizzard and ileum. Crop pH was lower ($p < 0.05$) in barley-fed birds than those fed wheat or sorghum, with rice producing an intermediate pH. Gizzard pH was similarly lower ($p < 0.05$) in barley-fed birds than those fed any of the other three cereals although sorghum-fed birds had a higher pH than those fed wheat with the rice causing a pH similar to both of these cereals. In the ileum, barley-fed birds had, again, a lower ($p < 0.05$) pH than those fed either

rice or sorghum. Wheat-fed birds had a similar ($p > 0.05$) ileal pH to all the other cereals. Duodenal, jejunal and colonic digesta pH and macerated kidney pH were not significantly altered ($p > 0.05$) by cereal type.

Table 25. Gut section digesta and kidney pH of birds euthanased after 48 h access to wheat, rice, sorghum and barley whole grain cold pelleted diets.

Gut section	Treatment	n	LS Mean	sd	min	Max	SE (LS mean)	P
CROP	Wheat	10	5.44 [b]	0.851	4.3	6.8	0.184	**0.040**
	Rice	9	5.29 [ab]	0.401	4.7	6.0	0.194	
	Sorghum	10	5.53 [b]	0.600	4.8	6.9	0.184	
	Barley	11	4.83 [a]	0.341	4.1	5.3	0.176	
GIZZARD	Wheat	12	3.98 [b]	0.305	3.5	4.5	0.097	**0.001**
	Rice	12	4.19 [bc]	0.396	3.7	4.9	0.097	
	Sorghum	12	4.34 [c]	0.373	3.6	4.9	0.097	
	Barley	12	3.38 [a]	0.255	3.0	3.9	0.097	
DUODENUM	Wheat	12	6.53	0.210	6.3	6.8	0.075	0.168
	Rice	12	6.31	0.360	5.3	6.7	0.075	
	Sorghum	12	6.49	0.207	6.3	6.9	0.075	
	Barley	12	6.40	0.237	5.9	6.8	0.075	
ILEUM	Wheat	9	8.83 [ab]	0.240	8.5	9.2	0.069	**0.007**
	Rice	8	8.95 [b]	0.169	8.7	9.1	0.073	
	Sorghum	7	9.04 [b]	0.151	8.8	9.2	0.078	
	Barley	12	8.70 [a]	0.226	8.1	9.0	0.059	
CAECUM	Wheat	11	6.42	0.820	5.5	7.8	0.182	0.165
	Rice	11	6.75	0.507	6.1	7.7	0.182	
	Sorghum	12	6.89	0.570	6.1	7.8	0.174	
	Barley	12	6.43	0.474	5.7	7.1	0.174	
COLON	Wheat	12	8.80	0.148	8.5	9.0	0.096	0.208
	Rice	12	8.53	0.456	7.3	9.2	0.096	
	Sorghum	12	8.70	0.252	8.0	9.0	0.096	
	Barley	11	8.57	0.395	7.5	9.0	0.101	
KIDNEY	Wheat	12	6.754	0.0705	6.62	6.85	0.0194	0.501
	Rice	12	6.769	0.0490	6.71	6.85	0.0194	
	Sorghum	12	6.777	0.0575	6.68	6.87	0.0194	
	Barley	12	6.795	0.0862	6.67	6.98	0.0194	

Plasma L- and D-lactic acid concentrations (Table 26) decreased ($p < 0.05$) from 0 to 24 h with a subsequent increase ($p < 0.05$) of the L-form to 48 h but not ($p > 0.05$) with the D- isomer. There was no significant influence ($p > 0.05$) from the grain type fed to the birds. The two isomers were related at the start and end of the trial i.e. higher levels of L-lactic indicated higher levels of D-lactic acid in the plasma. Plasma pH and L-lactic acid levels were related ($p < 0.05$) at 48 h i.e. plasma pH was higher in birds with higher L-lactic acid levels. This relationship was not sustained with the D-isomer nor at time 0.

Table 26. Influence of wheat, rice, sorghum or barley-based whole-grain diets on the plasma concentration of L- and D-lactic acid (mMol/L) of growers (n=12) at 14 weeks old.

Factor	Time (h)					
	0	24	48	0	24	48
	L-lactic acid			D-lactic acid		
Plasma concentration (mMol/L)	5.40 [a]	3.43 [c]	3.98 [b]	0.029 [a]	0.003 [b]	0.006 [b]
SE		0.153			0.0052	
Wheat	5.45	3.59	4.44	0.018	0.003	0.008
Rice	5.72	3.39	3.76	0.058	0.006	0.002
Sorghum	5.28	3.25	3.91	0.013	0	0.003
Barley	5.13	3.48	3.81	0.028	0.003	0.010
SE		0.378			0.0107	
Analysis						
Factor		P			P	
Time		**0.0001**			**0.0005**	
Feed		0.6001			0.2658	
Time* Feed		0.8871			0.2716	

Digesta L- and D-lactic acid concentrations (Table 27) were not significantly altered ($p > 0.05$) by cereal type in the ileum or caeca.

Table 27. Digesta L- and D-lactic acid concentration (mMol/L) in the crop, mid-jejunum, distal ileum and caeca of 14 week old female growers after 48 h access to a whole-grain, cold-pelleted, crumbled wheat, rice, sorghum or barley-based diet.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Crop	Wheat	12.80 [b]	4.077	**0.033**	8.18	5.362	0.070
	Rice	14.26 [b]	2.883		9.17	3.791	
	Sorghum	32.81 [a]	7.061		34.16	9.287	
	Barley	24.28 [a]	2.883		18.84	3.791	
Jejunum	Wheat	10.24 [ab]	1.203	**0.013**	0.54	0.193	0.070
	Rice	14.12 [a]	1.522		1.29	0.244	
	Sorghum	9.41 [b]	1.203		0.68	0.193	
	Barley	7.49 [b]	1.076		0.49	0.172	
Ileum	Wheat	5.17	1.001	0.301	0.31	0.446	0.549
	Rice	7.10	0.791		1.08	0.353	
	Sorghum	6.36	0.791		1.01	0.353	
	Barley	5.32	0.675		0.93	0.301	
Caeca	Wheat	1.40	0.421	0.077	1.28	0.365	0.087
	Rice	0.37	0.543		0.34	0.471	
	Sorghum	0.31	0.384		0.25	0.333	
	Barley	1.55	0.384		1.24	0.333	

The concentration of the L- isomer was greater ($p < 0.05$) in the crop when sorghum and barley were fed and lower ($p < 0.05$) in the jejunum with sorghum or barley diets compared with the rice diet; the wheat diet producing an intermediate concentration. The D- isomer was not significantly altered by grain type although the levels in the crop reflected the level of the L- isomer but at a reduced

proportion with the wheat and rice diets. It is worth noting the higher errors resulting from a high proportion of samples returning nil detection of D-lactic acid. A positive relationship between the L- and D- isomers was sustained ($p < 0.05$) in the digesta in each of the gut segments. However, there was no relationship ($p > 0.05$) across the gut segments for either isomer. Nor were relationships found ($p > 0.05$) between lactic acid content and digesta pH in any gut segment.

Jejunal short chain fatty acid concentration (mMol/L) (Table 28) was similar ($P > 0.05$) across feeds. Crop concentrations of acetic acid varied with grain type with wheat and rice producing a lower ($p < 0.05$) quantity than the barley and with sorghum producing an intermediate quantity. Heptanoic acid was less ($p < 0.05$) with the wheat and barley diets than rice and with sorghum similar to the other grain types. Total SCFA concentration was lower ($p < 0.05$) on the wheat diet than the barley and with the rice and sorghum diets producing an intermediate quantity to these. Ileal (distal ileum) concentrations of heptanoic acid were lower ($p < 0.05$) with the wheat, rice and sorghum diets than the barley. In the caecal digesta, wheat produced less ($p < 0.05$) propionic acid than the other grains. Barley produced a higher ($p < 0.05$) concentration of n-butyric and n-valeric acids than the other grains whilst *iso*-butyric acid was present ($p < 0.05$), at low concentration, on the rice and sorghum diets but not the wheat or barley. Total SCFA were higher ($p < 0.05$) with the barley diet than the rice and sorghum diets, with the wheat diet producing an intermediate quantity.

The relative proportions (%) of the total SCFA (Table 29) were not significantly altered ($p > 0.05$) by grain type in the jejunum or ileum digesta. In the crop, acetic acid was a lower ($p < 0.05$) proportion on the rice diet than the wheat or barley, whilst rice produced a higher ($p < 0.05$) proportion of heptanoic acid than the wheat or barley, with, for each SCFA, sorghum producing intermediate proportions. Caecal digesta SCFA proportions presented a complex result with acetic acid being lower ($p < 0.05$) on the barley diet than the other three grains. Propionic acid was similarly low ($p < 0.05$) on the wheat and barley diets, greater ($p < 0.05$) on the sorghum diet although similar to the barley diet, with rice producing a higher ($P < 0.05$) concentration. Rice produced proportionally less ($p < 0.05$) n-butyric acid than the wheat, barley a greater ($p < 0.05$) proportion again whilst the sorghum proportion was intermediate to the rice and wheat. No *iso*-butyric acid was detected on the wheat and barley diets while rice and sorghum diets contributed ($p < 0.05$) a minute proportion of this SCFA. Finally, n-valeric acid constituted a greater ($p < 0.05$) proportion of total SCFA on the barley diet than the other three cereals.

6.4 Discussion

The pattern established in the previous trials was maintained with similar concentrations of L- and D-lactic acids in the caeca but much lower concentrations of D- relative to the L-form in the other gut segments.

The digesta transit pattern for each cereal type may be determined from the relative concentrations of the lactic acid in each gut organ. In the crop wheat and rice produce less lactic acid than the sorghum and barley with the reverse in the jejunum. The structure and composition of the grains and the possible differences in starch granule disruption and gelatanisation with the crushing and moderate temperatures produced by cold-pelleting allied with the retrogradation upon cooling may all affect the starch entering each gut section. Constituents of the grain such as NSP, varying greatly in each cereal type, may affect the digestive process as well.

Table 28. Influence of whole-grain, cold-pelleted and crumbled wheat, rice, sorghum or barley-based diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the crop, mid-jejunum, distal ileum and caeca of 14 week old female growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)								
			Acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Crop	Wheat	9	**3.781** [b]	0	0.0028	0	0	0	0	**0.199** [b]	**3.983** [b]
	Rice	10	**3.941** [b]	0	0	0	0	0	0	**0.695** [a]	**4.636** [ab]
	Sorghum	5	**5.316** [ab]	0	0	0	0	0	0	**0.523** [ab]	**5.839** [ab]
	Barley	9	**5.908** [a]	0	0	0	0	0	0	**0.252** [b]	**6.161** [a]
	SE W B		0.5957		0.00147					0.1264	0.5633
	SE Rice		0.5651							0.1199	0.5344
	SE Sorghum		0.7992							0.1695	0.7558
	P		**0.049**		0.46					**0.028**	**0.044**
Jejunum	Wheat	12	0.516	0	0	0.023	0	0	0	0.040	0.579
	Rice	12	0.409	0	0	0.030	0	0	0	0.097	0.535
	Sorghum	11	0.569	0	0	0.065	0	0	0	0.019	0.652
	Barley	12	0.581	0	0	0.045	0	0	0	0.075	0.701
	SE W R B		0.0837			0.0129				0.0377	0.0994
	SE Sorghum		0.0874			0.0135				0.0394	0.1038
	P		0.46			0.14				0.49	0.65
Ileum	Wheat	12	0.487	0	0.0010	0	0	0	0	**0.122** [b]	0.293
	Rice	12	0.414	0	0	0	0	0	0	**0.109** [b]	0.370
	Sorghum	11	0.537	0	0	0	0	0	0	**0.134** [b]	0.390
	Barley	12	0.566	0	0	0	0	0	0	**0.221** [a]	0.412
	SE W R B		0.0569		0.00048					0.0183	0.0702
	SE Sorghum		0.0595							0.0192	0.0733
	P		0.27		0.42					**0.0003**	0.056
Caeca	Wheat	12	35.538	**2.454** [b]	**5.807** [b]	**0** [b]	**0.553** [b]	0.010	0	0	**44.361** [ab]
	Rice	12	27.951	**3.805** [a]	**2.632** [b]	**0.118** [a]	**0.452** [b]	0.120	0	0	**35.078** [b]
	Sorghum	12	34.199	**3.849** [a]	**4.190** [b]	**0.152** [a]	**0.450** [b]	0.097	0	0	**42.937** [b]
	Barley	12	41.528	**4.278** [a]	**10.934** [a]	**0** [b]	**1.314** [a]	0	0	0	**58.053** [a]
	SE		3.9272	0.3985	1.1984	0.0412		0.0392			5.1401
	P		0.13	**0.014**	**0.0001**	**0.019**	**0.0001**	0.08			**0.024**

Table 29. Influence of feeding a whole-grain, cold-pelleted, crumbled wheat, rice, sorghum or barley-based diet on the individual short chain fatty acid proportion (%) of total short chain fatty acid in the digesta content in the crop, mid-jejunum, distal ileum and caeca of 14 week old female growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Crop	Wheat	9	**0.954** [a]	0	0.0006	0	0	0	0	**0.046** [b]
	Rice	10	**0.823** [b]	0	0	0	0	0	0	**0.177** [a]
	Sorghum	5	**0.905** [ab]	0	0	0	0	0	0	**0.095** [ab]
	Barley	9	**0.950** [a]	0	0	0	0	0	0	**0.050** [b]
	SE W B		0.0336		0.00033					0.0335
	SE Rice		0.0318							0.0318
	SE Sorghum		0.0450							0.0449
	P		**0.027**		0.46					**0.027**
Jejunum	Wheat	12	0.898	0	0	0.031	0	0	0	0.070
	Rice	12	0.758	0	0	0.044	0	0	0	0.115
	Sorghum	11	0.827	0	0	0.070	0	0	0	0.103
	Barley	12	0.830	0	0	0.059	0	0	0	0.111
	SE W R B		0.0665			0.0126				0.0610
	SE Sorghum		0.0694			0.0131				0.0637
	P		0.53			0.17				0.95
Ileum	Wheat	12	0.790	0	0.0012	0	0	0	0	0.209
	Rice	12	0.689	0	0	0	0	0	0	0.228
	Sorghum	11	0.795	0	0	0	0	0	0	0.205
	Barley	12	0.732	0	0	0	0	0	0	0.268
	SE W R B		0.0447		0.00059					0.0334
	SE Sorghum		0.0467							0.0348
	P		0.30		0.42					0.54
Caeca	Wheat	12	**0.808** [a]	**0.063** [c]	**0.116** [b]	**0** [b]	**0.012** [b]	0.0003	0	0
	Rice	12	**0.795** [a]	**0.114** [a]	**0.072** [c]	**0.003** [a]	**0.012** [b]	0.0041	0	0
	Sorghum	12	**0.799** [a]	**0.091** [b]	**0.094** [bc]	**0.004** [a]	**0.010** [b]	0.0025	0	0
	Barley	12	**0.715** [b]	**0.076** [bc]	**0.187** [a]	**0** [b]	**0.022** [a]	0	0	0
	SE		0.0161	0.0074	0.0147	0.0011	0.0019			
	P		**0.0005**	**0.0001**	**0.0001**	**0.035**	**0.0001**	0.09		

The quantity and type of endogenous grain α-amylase and differences in the resistance of each cereals' starch to the birds' amylase(s) may similarly affect the pattern of fermentation even without considering the initial effects on the micro-organism populations in the gut sections of each cereal type.

The 48 h lactic acid (D-lactate particularly) and VFA concentrations were lower and pH higher than in the previous trial when ground diets were fed. Further experimental work to directly compare the effects of feed processing is warranted given these results. With the tendency for coarser diets to be held for longer in the upper gut (Taylor, 1998 and see broiler trials below) perhaps more complete digestion has occurred and reduced the material available for fermentation in the lower ileum and hindgut. This is contradicted somewhat by the lactate levels found in the ileum, marginally higher with the rice and sorghum diets than the wheat and barley but with these latter grains producing, at least numerically, more lactate in the caeca. However, relationships between plasma pH and plasma lactate (R^2 = 0.221) and caecal pH and total organic acids in the caeca (R^2 = 0.416) were weak. Replication appears to be one weakness in this work. Moreover, individual birds display some extremes of both pH and VFA and lactate production which indicates some greater effect of fermentation in individual animals. This may be due to different microbial populations established in each animal or may indicate that individual response to sudden dietary change require variable adjustment periods in the individual for a host of reasons. Similar propositions of responses of individual birds have been made in relation to many diet related production problems e.g. the "low AME diet or low AME bird" proposition.

There was little evidence of a hindgut fermentation problem being evident in the current results. There was substantial D-lactate produced in the crop of sorghum and barley fed birds which may indicate different microbial loads on these cereals or responses of resident populations of organisms in the crop to constituents of these cereals. The basic role of the crop is to allow grain to soften and swell (Appleby *et al*., 1992) and the rate of crop emptying depends on particle size and moistening (Larbier and Lecelerq, 1992). There may be some difference between grains of different original sizes in how they are broken and crushed through the same sized die. Some of the above differences between the cereals may have been caused by these physical effects but, irrespectively, plasma lactate levels did not indicate substantial accumulation of D-lactate in the birds.

The difference in feed intakes from d 1 to d 2, whilst being significantly different, were of small magnitude being 7 percent lower on d2. As grain type did not affect this result it was not considered to be of consequence particularly as the birds were eating feed at the breeders'recommended intakes and specifications.

In the first four experiments the birds were all quite young and had relatively small feed intakes. The feed consumption in the laying period, particularly around peak production, would be substantially greater and perhaps influence digesta passage and responses differently.

7. Comparison of wheat, rice, sorghum and barley-based cold-pelleted, crumbled diets on digesta factors in layers at peak production.

7.1 Introduction

The previous experiments had been performed with growers in the mid-rearing period. In each trial feed intake was limited as normally found in layer types. In fact, the NRC (1994) indicated the concern for maintaining adequate feed intake in the modern, feed efficient, small-bodied bird particularly with inclusion of ingredients that could cause dietary dilution. Leeson *et al*, (1993) indicated that young laying birds were not very adept at balancing energy intakes and feed intake could be a problem. There would be an expectation of substantially greater feed intake with heavy egg production and, hence, effects of fermentation in the lower gut may occur in periods of the production cycle when digesta content and passage rate may contribute to favourable conditions for such a process.

For the reasons described above, the following experiment was conducted to monitor the effects of four different cereal diets on gut function in birds in the immediate post-peak production period.

7.2 Materials and methods

As per General Materials and Methods (above). The birds had been fed through lay on commercial layer rations (supplied by Weston Animal Nutrition). At 168 d old, the birds were allocated to treatments (n=12) at lights-on on d 1 after 5 d in the experimental cages. Feeds were prepared as layer diets as specified (General Materials and Methods) and cold-pelleted and crumbled.

7.3 Results

Feed intake (g) was not influenced ($p > 0.05$) by grain type (78.6, 85.0 ± 4.12 g, 79.9 and 90.5 ± 4.30 g for wheat, rice, sorghum and barley respectively) nor ($p > 0.05$) from d1 to d2 (82.7 and 84.3. ± 2.98 g).

Excreta pH (Table 30) from 12 to 48 h was influenced by the excreta pH at time 0 (the pH at time 0 is included in the table for comparative purposes although, as a significant covariate, it is excluded from the repeated measures comparison).

Excreta pH decreased ($p < 0.05$) from 12 to 24 h, maintained a similar ($p > 0.05$) value at 36 h then decreased ($p < 0.05$) again at 48 h. Wheat produced a higher ($p < 0.05$) pH than rice and barley, sorghum a higher ($p < 0.05$) pH than rice but similar to barley, while rice and barley had similar excreta pH (6.94^{a}, 6.56^{c} ± 0.090, 6.86^{ab} and 6.67^{bc} ± 0.094 for wheat, rice, sorghum and barley respectively). Excreta pH was not affected ($p > 0.05$) by the quantity of feed intake.

Caecal evacuation pH, measured at 0, 12 and 24 h, only differed ($p < 0.05$) from 12 to 24 h (6.35^{ab} ± 0.117, 6.11^{b} ± 0.109 and 6.56^{a} ± 0.136) but did not alter significantly ($p > 0.05$) with cereal type (6.20 ± 0.168, 6.53 ± 0.113, 6.42 ± 0.141 and 6.21 ± 0.132 for wheat, rice, sorghum and barley respectively).

Table 30. Influence of wheat, rice, sorghum or barley-based cold-pelleted, crumbled diets on the fresh excreta pH of layers (n=12) at peak production at 24 weeks old.

Factor	Time (h)				
	0	12	24	36	48
Mean pH	7.29	7.49 [a]	6.87 [b]	6.75 [b]	5.93 [c]
SE					
Wheat	7.28	7.35	7.26	6.90	6.26
Rice	7.34	7.40	6.50	6.54	5.79
Sorghum	7.12	7.85	6.85	6.93	5.79
Barley	7.44	7.34	6.86	6.62	5.87
SE					

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	**0.0031**
Feed	**0.0137**	Feed intake d 1	0.2152
Time*Feed	0.2617		

As with excreta pH, plasma pH was affected ($p < 0.05$) by the initial measure for each bird. Plasma pH (Table 31) decreased ($p < 0.05$) from 24 to 48 h but was not altered ($p > 0.05$) by grain type (7.617 ± 0.0102, 7.636 ± 0.0104, 7.636 ± 0.0109 and 7.615 ± 0.0106 for wheat, rice, sorghum and barley respectively). Plasma pH was not influenced ($p > 0.05$) by feed intake on d 1 nor by excreta pH.

Table 31. Influence of wheat, rice, sorghum or barley-based whole-grain diets on the chilled plasma pH of layers (n=12) at peak production at 24 weeks old.

Factor	Time (h)		
	0	24	48
Mean pH	7.660	7.662 [a]	7.591 [b]
SE		0.0075	0.0073
Wheat	7.652	7.659	7.575
Rice	7.656	7.668	7.604
Sorghum	7.665	7.669	7.603
Barley	7.658	7.650	7.581
SE		0.0157	0.0150

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	0.8931
Feed	0.3174	Feed intake d 1	0.4773
Time*Feed	0.9073	Plasma pH Time 0	**0.0001**

After 48 h on-feed, gut section digesta and macerated kidney pH (Table 32) revealed differences ($p < 0.05$) across grain type in the gizzard, ileum and colon. Gizzard pH was lower ($p < 0.05$) in barley-fed birds than those fed the other three cereals. In the ileum, rice-fed birds had a lower ($p < 0.05$) digesta pH than those fed the other cereals. Wheat-fed birds had a higher ($p < 0.05$) colon digesta pH than birds fed rice or sorghum but with barley intermediate between the two groups. Crop, duodenal and caecal digesta and macerated kidney pH were unaltered ($p > 0.05$) by grain type.

Table 32. Gut section digesta and kidney pH of layers euthanased after 48 h access to wheat, rice, sorghum and barley cold-pelleted, crumbled diets at peak production at 24 weeks.

Gut section	Treatment	N	LS Mean	Sd	Min	max	SE (LS mean)	P
CROP	Wheat	9	4.74	0.45	4.2	5.6	0.133	0.975
	Rice	8	4.69	0.40	4.1	5.4	0.141	
	Sorghum	9	4.76	0.33	4.2	5.2	0.133	
	Barley	8	4.69	0.41	4.2	5.2	0.141	
GIZZARD	Wheat	12	4.35 [a]	0.28	3.7	4.8	0.115	**0.002**
	Rice	11	4.22 [a]	0.52	3.0	4.7	0.121	
	Sorghum	11	4.17 [a]	0.41	3.6	4.9	0.121	
	Barley	11	3.69 [b]	0.37	3.1	4.3	0.121	
DUODENUM	Wheat	12	6.08	0.22	5.8	6.5	0.090	0.150
	Rice	12	5.87	0.40	4.9	6.3	0.090	
	Sorghum	11	6.09	0.37	5.1	6.4	0.094	
	Barley	11	6.15	0.21	5.7	6.4	0.094	
ILEUM	Wheat	9	7.99 [a]	0.28	7.5	8.4	0.184	**0.015**
	Rice	6	7.03 [b]	1.07	5.2	8.0	0.225	
	Sorghum	11	7.67 [a]	0.48	6.8	8.3	0.166	
	Barley	11	7.85 [a]	0.36	7.0	8.3	0.166	
CAECUM	Wheat	12	6.21	0.46	5.4	7.0	0.122	0.153
	Rice	11	6.60	0.28	6.1	6.9	0.128	
	Sorghum	11	6.38	0.58	5.2	7.0	0.128	
	Barley	9	6.52	0.26	6.1	6.9	0.141	
COLON	Wheat	12	7.60 [a]	0.85	6.2	8.5	0.206	**0.022**
	Rice	10	6.62 [b]	0.61	5.7	7.8	0.226	
	Sorghum	10	6.97 [b]	0.52	6.0	7.6	0.226	
	Barley	11	7.05 [ab]	0.78	5.7	7.9	0.216	
KIDNEY	Wheat	12	6.879	0.091	6.67	6.98	0.0251	0.274
	Rice	12	6.912	0.094	6.72	7.03	0.0251	
	Sorghum	11	6.929	0.085	6.75	7.06	0.0262	
	Barley	11	6.949	0.075	6.84	7.05	0.0262	

Plasma L- and D-lactic acid concentrations (Table 33) were unaltered ($p > 0.05$) from 0 to 48 h despite the decline in the L-form approaching significance ($p = 0.052$). Grain type did not significantly influence ($P > 0.05$) either isomer although the increase in D-lactic acid in the rice-fed birds over the 48 h again approached significance ($p = 0.082$). The errors associated with the D-isomer were very large, indicating the greater variation in levels of D-lactic acid found in individual birds.

Digesta L- and D-lactic acid concentrations (Table 34) were not significantly altered ($p > 0.05$) by cereal type in the crop or caeca but were greater ($p < 0.05$) in the jejunum and ileum with the rice diet although, in the jejunum, a similarly high L-lactic acid concentration was produced on the sorghum diet. The D- isomer was produced at a similar level in the crop and caeca, much less in the ileum but at approximately 2/3 to ½ that of the L-isomer except when the rice diet was fed. The D-isomer on the rice diet was a proportionally greater amount of the L-form in the jejunum and was actually produced in a concentration in excess of the L-isomer in the crop and ileum.

Table 33. Influence of wheat, rice, sorghum or barley-based diets on the plasma concentration of L- and D-lactic acid (mMol/L) of layers (n=12) at peak production at 24 weeks old.

Factor	Time (h)					
	0	24	48	0	24	48
	L-lactic acid			D-lactic acid		
Plasma concentration (mMol/L)	5.40	5.09	4.54	0.094	0.075	0.140
SE		0.249			0.0437	
Wheat	6.31	4.79	4.95	0.118	0.053	0.043
Rice	5.04	5.42	4.83	0.008	0.128	0.407
Sorghum	5.01	5.16	4.10	0.098	0.077	0.058
Barley	5.23	4.98	4.29	0.150	0.043	0.053
SE		0.510			0.0893	

Analysis Factor	P	P
Time	0.0515 *	0.5597
Feed	0.4498	0.3520
Time* Feed	0.5426	0.0815

* Note significance value

Table 34. Digesta L- and D-lactic acid concentration (mMol/L) in the crop, mid-jejunum, distal ileum and caeca of 24 week old layers at peak production after 48 h access to cold-pelleted, crumbled wheat, rice, sorghum or barley-based diets.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Crop	Wheat	20.27	3.043	0.98	21.60	5.415	0.44
	Rice	20.66	2.722		30.93	4.843	
	Sorghum	20.04	2.152		22.54	3.829	
	Barley	19.41	1.925		21.80	3.425	
Jejunum	Wheat	6.82 [b]	1.387	**0.014**	0.83 [b]	1.509	**0.024**
	Rice	13.61 [a]	1.498		7.65 [a]	1.630	
	Sorghum	9.55 [ab]	1.835		1.75 [b]	1.996	
	Barley	7.03 [b]	1.498		1.17 [b]	1.630	
Ileum	Wheat	9.03 [b]	2.170	**0.007**	6.49 [b]	2.863	**0.002**
	Rice	17.83 [a]	1.913		18.56 [a]	2.525	
	Sorghum	9.58 [b]	2.567		4.74 [b]	3.388	
	Barley	7.45 [b]	2.344		3.61 [b]	3.093	
Caeca	Wheat	2.12	0.718	0.54	1.96	0.698	0.62
	Rice	2.52	0.900		2.41	0.875	
	Sorghum	0.91	0.793		0.93	0.771	
	Barley	1.57	0.793		1.62	0.771	

Short chain fatty acid concentration (mMol/L) (Table 35) and relative proportions of total SCFA (Table 36) in the digesta in each gut segment were similar ($P > 0.05$) across feeds.

Table 35. Influence of cold-pelleted, crumbled wheat, rice, sorghum or barley-based diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the crop, mid-jejunum, distal ileum and caeca of 24 week old layers at peak production.

Organ	Diet	N	Short chain fatty acid (C1-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Crop	Wheat	7	7.753	0	0	0.006	0.122	0	0	0.007	7.889
	Rice	9	7.665	0	0	0.025	0.014	0	0	0.040	7.744
	Sorghum	10	12.535	0	0	0.038	0	0	0	0.051	12.624
	Barley	10	10.795	0	0	0.012	0.125	0	0	0.015	10.947
	SE Wheat		1.8485			0.0105	0.0641			0.0162	1.8683
	SE Rice		1.6302			0.0092	0.0565			0.0143	1.6477
	SE S B		1.5465			0.0088	0.0536			0.0136	1.5632
	P		0.11			0.10	0.25			0.13	0.12
Jejunum	Wheat	10	0.777	0	0	0.367	0	0	0	0.002	1.146
	Rice	11	1.209	0	0	0.500	0	0	0	0	1.709
	Sorghum	9	1.092	0	0	0.532	0	0	0	0	1.624
	Barley	10	1.086	0	0	0.484	0	0	0	0	1.570
	SE W B		0.1509			0.0482				0.0009	0.1805
	SE Rice		0.1439			0.0460					0.1721
	SE Sorghum		0.1590			0.0508					0.1903
	P		0.22			0.11				0.40	0.14
Ileum	Wheat	11	0.819	0.016	0.012	0.066	0.005	0.007	0.0105	0.005	0.940
	Rice	11	1.599	0.002	0	0.097	0	0	0.0001	0.003	1.701
	Sorghum	9	1.219	0.040	0	0.096	0	0	0.0200	0.014	1.389
	Barley	9	1.125	0.005	0.003	0.049	0	0	0.0034	0	1.186
	SE W R		0.1977	0.0190	0.0063	0.0292	0.0025	0.0036	0.01028	0.0055	0.2147
	SE S B		0.2185	0.0210	0.0070	0.0322			0.01137	0.0061	0.2374
	P		0.065	0.56	0.54	0.64	0.47	0.47	0.59	0.42	0.10
Caeca	Wheat	12	21.693	1.740	3.259	0.024	0.433	0.024	0	0	27.173
	Rice	12	23.762	2.989	3.558	0.040	0.575	0.040	0	0.003	30.967
	Sorghum	11	32.594	3.203	5.806	0.107	0.475	0.075	0	0	42.260
	Barley	11	24.528	2.229	5.706	0.023	0.567	0.047	0	0	33.100
	SE W R		3.6449	0.4291	1.1240	0.0391	0.1379	0.0344		0.0013	4.7857
	SE S B		3.8070	0.4482	1.1739	0.0408	0.1441	0.0359			4.9984
	P		0.20	0.08	0.26	0.43	0.86	0.77		0.43	0.18

Table 36. Influence of feeding cold-pelleted, crumbled wheat, rice, sorghum or barley-based diets on the individual short chain fatty acid proportion (%) of total short chain fatty acid in the digesta content in the crop, mid-jejunum, distal ileum and caeca of 24 week old layers at peak production.

Organ	Diet	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Crop	Wheat	7	0.987	0	0	0.0006	0.012	0	0	0.0007
	Rice	9	0.985	0	0	0.0046	0.001	0	0	0.0090
	Sorghum	10	0.992	0	0	0.0032	0	0	0	0.0048
	Barley	10	0.989	0	0	0.0014	0.008	0	0	0.0011
	SE Wheat		0.0066			0.00177	0.0051			0.00326
	SE Rice		0.0058			0.00156	0.0045			0.00287
	SE S B		0.0055			0.00148	0.0042			0.00273
	P		0.86			0.30	0.23			0.18
Jejunum	Wheat	10	0.668	0	0	0.331	0	0	0	0.001
	Rice	11	0.689	0	0	0.311	0	0	0	0
	Sorghum	9	0.675	0	0	0.325	0	0	0	0
	Barley	10	0.680	0	0	0.320	0	0	0	0
	SE W B		0.0244			0.0246				0.0007
	SE Rice		0.0232			0.0235				
	SE Sorghum		0.0257			0.0260				
	P		0.94			0.95				0.40
Ileum	Wheat	11	0.856	0.014	0.010	0.096	0.004	0.006	0.009	0.004
	Rice	11	0.932	0.003	0	0.063	0	0	0	0.001
	Sorghum	9	0.922	0.016	0	0.048	0	0	0.008	0.006
	Barley	9	0.958	0.003	0.002	0.034	0	0	0.002	0
	SE W R		0.0407	0.0103	0.0055	0.0280	0.0022	0.0032	0.0061	0.0030
	SE S B		0.0450	0.0114	0.0061	0.0310			0.0068	0.0033
	P		0.37	0.75	0.52	0.48	0.47	0.47	0.67	0.55
Caeca	Wheat	12	0.786	0.074	0.125	0.0007	0.014	0.0007	0	0
	Rice	12	0.791	0.095	0.097	0.0013	0.015	0.0012	0	0.0001
	Sorghum	11	0.783	0.078	0.126	0.0021	0.009	0.0015	0	0
	Barley	11	0.745	0.084	0.153	0.0008	0.016	0.0024	0	0
	SE W R		0.0248	0.0120	0.0187	0.00101	0.0034	0.00110		0.00004
	SE S B		0.0259	0.0125	0.0195	0.00106	0.0036	0.00115		
	P		0.56	0.63	0.25	0.77	0.55	0.73		0.43

7.4 Discussion

D-lactic acid produced in excess of the L-form indicates accumulation and is associated with lactic acidosis in other animals and humans particularly so with the levels found in the ileum in this experiment. Jejunal lactic acid may be produced as an end product of glycolysis rather than fermentation but fermentative production cannot be discounted as some SCFA was detected in the jejunum as well. In humans, SCFA in the jejunum have been considered to be due to migration of anaerobes up the ileum *post mortem* (H. Butt, Senior Research Scientist, Hunter Area Pathology Services, *pers. comm*.) but in these experiments the samples were taken from the birds within 3 min of death and were stripped caudally after a stream of clear digesta was discarded. The samples were placed immediately on ice, so minimising, if not preventing, contamination derived fermentation.

The lactic acid levels were elevated in all the lower gut segments over those found in earlier trials. The lactate production in the lower ileum on the rice diet was sufficient to be described as indicative of lactic acidosis; lactate is not a key intermediate in fermentation and little is usually found in the gut but when flux through glycolysis is high when large quantities of substrate flow through the gut, lactate production is favoured (Cumming, 1981). Although plasma levels were not greatly elevated, there was a correlation between plasma and lower gut concentrations to indicate potential problems. The lactate levels were not replicated in VFA concentrations found in the different gut sections. VFA concentrations and proportions were generally lower than or similar to those found in the previous experiments. Of some interest was the same consistent value for propionic acid production in this experiment as in earlier trials. The expectation would be that at least some propionate would be produced if lactate concentrations were elevated. However, lactate would only be converted to propionate if the appropriate micro-organisms were present. If substantial quantities of carbohydrate were being converted to pyruvate thence to the TCA cycle, with overload, NAD/NADH, lactate is produced i.e. there is a need for H^+ "sinks" (S. Bird, *pers.comm*.).

Although digesta pH was not greatly lower than in previous trials some indication of more acid production was evident with a lower ileal pH on the rice diet and excreta pH decreased substantially from initial levels at 24 and 36 h and again at 48 h. Plasma pH was also reduced at 48 h indicating some reduction in the buffering ability of the blood with, it is presumed, an increasing acid load.

The above responses could be explained by a greater feed intake required with heavy production; the digestive process would be more active both because of nutritive demand and quantity of digesta present. The rate of feed passage may be hastened to meet these demands. It is possible that more starch may be entering the lower tract and, with the effects of adjustment to a feed change, fermentation may be encouraged. The lactate concentration may indicate that ileal fermentation was commencing but that it may take more than 48 h for microbial populations to increase and/or be changed to then increase VFA production. Thus, greatly increased lactate concentrations may be an indicator of a greater fermentation to be stimulated over a longer period. Alternatively, of course, the effect may well be transitory and VFA levels may not increase over the longer period.

An additional point was that there was a period of heat stress for the birds over the course of this experiment. Although shed temperatures did not exceed 35 ºC (the birds were housed in an insulated room within the insulated shed but with tunnel ventilation of external air without cooling) the birds had been used to mild conditions throughout the rearing phase. The birds were drinking substantial amounts of water (not measured) and some diarrhoea was present in most birds. This is an important observation in itself as feed passage may be hastened by a more fluid digesta and lead to a fermentation of feed constituents moved further down the digestive tract. The point to make is that starch digestion may be moved to a different site in the gut through the effects of many factors; some of which may take effect very quickly.

8. Digesta conditions in growers fed a commercial diet or a wheat-based diet with or without enzyme.

8.1 Introduction

The use of exogenous feed enzymes in broiler diets, notably those that are wheat or barley-based, allows for improved dietary utilisation principally via a reduction in digesta viscosity which is well correlated with improved feed conversion efficiency (Bedford, 1996). Recently there has been active promotion of and application of exogenous enzymes to layer diets with improved feed conversion efficiency again being the major benefit (Bird, 1996). As all dietary components, including fat, are better digested, a viscosity rather than a cell-wall based degradation mechanism has been suggested for this improvement (Bedford, 1996).

As pH influences enzyme activity (Marquardt and Bedford, 1996), the effect of a change of diet or the diet base on digesta factors including pH may affect the performance of an exogenous enzyme. Alternatively, the added enzyme may change digesta characteristics and performance advantages may accrue.

The following experiment examined the effects on digesta when a wheat-based diet, either with or without an exogenous feed enzyme, was substituted for the birds' standard commercial ration.

8.2 Materials and methods

A second group of birds (again AZTEC 101 / 007) were reared from day-old as per General Materials and Methods (above). The birds had been fed on a commercial steam-pelleted, crumbled starter and grower rations supplied by an alternative miller. At 105 d old, the birds were allocated to treatments (n=12) at lights on on d 1 after 5 d in the experimental cages. Treatment 1 had the birds continuing on the commercial ration, Treatments 2 and 3 were wheat-based, cold-pelleted and crumbled diets with or without a commercial enzyme (Bio-Feed Wheat) incorporated in the mix at 200g tonne^{-1}. The wheat was the then current season but aged grain at an equivalent stage to that used in the previous trials. The wheat was sourced from the alternative miller.

At slaughter, additional samples of approximately 1 g of both ileal and caecal digesta were collected and dried for 24 h in a fan-forced oven at 80ºC. After equilibrating at ambient temperature, the moisture content was used to calculated SCFA content in the digesta on a DM basis.

8.3 Results

Feed intake (g) was not influenced ($p > 0.05$) by feed (51.1, 56.5 and 60.8 ± 3.57 g for commercial, wheat and wheat + E respectively) nor ($p > 0.05$) from d1 to d2 (54.2 and 58.1 ± 2.91 g).

Excreta pH (Table 37) decreased ($p < 0.05$) from 12 to 24 h then returned to the original level at 36 h. It is of note that the 36 h pH was greater ($p < 0.05$) than that at 48 h. Wheat diets reduced ($p < 0.05$) excreta pH irrespective of enzyme application (7.64^{a}, 7.26^{b} and 7.21^{b} ± 0.069 for commercial, wheat and wheat + E feeds respectively). Excreta pH was not affected ($p > 0.05$) by the excreta or plasma pH at time 0 nor by feed intake.

Table 37. Influence of commercial, wheat or wheat + E crumbled diets on the fresh excreta pH of growers (n=12) at 15 weeks old.

Factor	Time (h)				
	0	12	24	36	48
Mean pH	7.41 [ab]	7.47 [ab]	6.95 [c]	7.66 [a]	7.37 [b]
SE			0.091		
Commercial	7.44	7.76	7.29	7.86	7.87
Wheat	7.48	7.52	6.72	7.61	6.97
Wheat + E	7.32	7.12	6.85	7.51	7.26
SE			0.158		

Analysis		Covariate analysis	
Factor	P	Factor	P
Time	**0.0001**	Excreta pH Time 0	NS nil convergence
Feed	**0.0001**	Feed intake d 1	NS nil convergence
Time*Feed	0.0852	Plasma pH Time 0	0.6733

Caecal evacuation pH was greater ($p < 0.05$) than the original 0 h value at 24 and 36 h and increased ($P < 0.05$) on the wheat diet compared with the wheat + E feed ($6.53^{ab} \pm 0.069$, $6.72^{b} \pm 0.075$ and $6.45^{a} \pm 0.069$ for commercial, wheat and wheat + E feeds respectively).

Plasma pH (Table 38) decreased ($p < 0.05$) each 24 h from 0 h to 48 h but was not altered ($p > 0.05$) by diet (7.764 ± 0.0086, 7.749 ± 0.0087 and 7.744 ± 0.0086 for commercial, wheat and wheat + E respectively).

Table 38. Influence of commercial, wheat and wheat + E crumbled diets on the chilled plasma pH of growers (n=12) at 15 weeks old.

Factor	Time (h)		
	0	24	48
Mean pH	7.792 [a]	7.750 [b]	7.715 [c]
SE	0.0086	0.0087	0.0086
Commercial	7.793	7.769	7.729
Wheat	7.799	7.742	7.705
Wheat + E	7.783	7.740	7.710
SE	0.0148	0.0155	0.0148

Analysis	
Factor	P
Time	**0.0001**
Feed	0.2564
Time*Feed	0.7895

The gut section digesta and macerated kidney pH (Table 39) did not differ ($p > 0.05$) across feed type.

Plasma L- lactic acid concentration (Table 40) decreased ($P < 0.05$) at 24 h relative to 0 and 48 h. D-lactic acid concentration did not alter ($p > 0.05$) from 0 to 48 h. Diet did not significantly influence ($P > 0.05$) either L- or D-lactic acid concentration.

Table 39. Gut section digesta and kidney pH of 15 week old growers euthanased after 48 h access to a commercial crumbled grower or a cold-pelleted, crumbled wheat or wheat plus enzyme diet.

Gut section	Treatment	n	LS Mean	sd	min	max	SE (LS mean)	P
GIZZARD	Commercial	12	3.91	0.51	3.1	4.5	0.161	0.954
	Wheat	12	3.85	0.60	3.0	4.9	0.161	
	Wheat + E	12	3.90	0.57	2.8	4.7	0.161	
ILEUM	Commercial	12	8.91	0.22	8.5	9.3	0.069	0.257
	Wheat	12	8.78	0.27	8.3	9.2	0.069	
	Wheat + E	12	8.76	0.22	8.4	9.1	0.069	
CAECUM	Commercial	12	6.04	0.79	5.4	7.7	0.182	0.759
	Wheat	12	6.07	0.48	5.2	7.3	0.182	
	Wheat + E	12	5.90	0.58	5.3	7.1	0.182	
COLON	Commercial	12	8.90	0.10	8.8	9.1	0.053	0.230
	Wheat	10	8.77	0.20	8.3	9.1	0.058	
	Wheat + E	10	8.84	0.23	8.6	9.4	0.058	
KIDNEY	Commercial	12	6.823	0.122	6.67	7.14	0.0295	0.719
	Wheat	12	6.850	0.082	6.78	7.07	0.0295	
	Wheat + E	12	6.818	0.099	6.70	7.00	0.0295	

Table 40. Influence of commercial, wheat or wheat + E feeds on the plasma concentration of L- and D-lactic acid (mMol/L) of growers (n=12) at 15 weeks old.

Factor	Time (h)					
	0	24	48	0	24	48
	L-lactic acid			D-lactic acid		
Plasma concentration (mMol/L)	5.15 [a]	4.18 [b]	5.50 [a]	0.017	0.018	0.024
SE	0.223	0.219	0.213	0.0049	0.0048	0.0047
Commercial	5.01	4.17	5.30	0.018	0.018	0.026
Wheat	4.85	4.54	5.84	0.016	0.010	0.033
Wheat + E	5.59	3.84	5.35	0.017	0.025	0.012
SE	0.404	0.386	0.369	0.0089	0.0085	0.0081

Analysis		
Factor	P	P
Time	**0.0001**	0.5681
Feed	0.7103	0.9300
Time* Feed	0.3966	0.2837

Digesta L- and D-lactic acid concentrations (Table 41) were not significantly altered ($p > 0.05$) by cereal type in the distal ileum or colon but were greater ($p < 0.05$) in the caeca when the wheat was fed with the enzyme supplemented wheat diet producing concentrations between the commercial and un-supplemented wheat diets. The D-isomer was produced in equivalent amounts to the L-form in the caeca and in substantially lower relative amounts in the ileum, as in the previous experiments, and in the colon.

A further repeated measures analysis of variance of lactic acid concentration along the three gut sections indicated that no differences ($p > 0.05$) in L-lactic acid production occurred from the distal ileum to the colon but the D-isomer was produced in similar ($p > 0.05$) amounts in the distal ileum and colon but in greater ($P < 0.05$) amounts in the caeca.

Table 41. Digesta L- and D-lactic acid concentration (mMol/L) in the distal ileum, caeca and colon of 15 week old growers after 48 h access to commercial crumbled or wheat or wheat + E cold-pelleted, crumbled diets.

Gut section	Feed	L-lactic acid			D-lactic acid		
		LS Mean	SE	p	LS Mean	SE	p
Ileum	Commercial	6.08	2.744	0.64	0.75	1.166	0.38
	Wheat	9.70			3.02		
	Wheat + E	7.28			1.41		
Caecum	Commercial	3.20 [b]	2.372	**0.029**	3.15 [b]	2.340	**0.032**
	Wheat	13.70 [a]	2.835		13.38 [a]	2.797	
	Wheat + E	9.23 [ab]	2.652		8.99 [ab]	2.617	
Colon	Commercial	8.55	1.886	0.23	1.76	0.579	0.79
	Wheat	3.82	1.886		1.17	0.647	
	Wheat + E	5.46	1.722		1.48	0.610	

Digesta dry matter (Table 42) was not affected ($p > 0.05$) by feed type and was similar ($P > 0.05$) in both the ileum and caeca (0.795 and 0.793 ± 0.0044 respectively).

Table 42. Digesta dry matter (%) in the ileum and caeca of 15 week old growers euthanased after 48 h access to a commercial crumbled grower or a cold-pelleted, crumbled wheat or wheat plus enzyme diet.

Gut section	Treatment	n	LS Mean	min	max	SE (LS mean)	P
ILEUM	Commercial	12	0.796	0.769	0.846	0.0077	0.241
	Wheat	12	0.802	0.775	0.826	0.0077	
	Wheat + E	12	0.788	0.765	0.821	0.0077	
CAECUM	Commercial	12	0.787	0.701	0.827	0.0077	0.241
	Wheat	12	0.801	0.743	0.847	0.0077	
	Wheat + E	12	0.790	0.746	0.835	0.0077	

Short chain fatty acid concentration (mMol/L) and the relative proportions (%) of each individual SCFA of the total SCFA (Tables 43 and 44 respectively) in the ileal and colon digesta were similar ($P > 0.05$) across feeds and correction for moisture content did not alter ($p > 0.05$) results for the ileal digesta. In the caecal digesta, moisture correction resulted in propionic acid concentration being significantly greater ($P < 0.05$) in birds fed the wheat diet than those fed the commercial or wheat + E diets. Further, in the caecal digesta, when calculated as log transformed data, the proportion of n-butyric acid of total SCFA was greater ($P < 0.05$) in birds fed the wheat + E rather than the wheat diet but similar ($p > 0.05$) to birds fed the original commercial diet.

Table 43. Influence of a commercial crumbled grower or a cold-pelleted, crumbled wheat or wheat plus enzyme diet on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the distal ileum, caeca and colon of 15 week old growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Commercial	12	6.940	0.124	0.027	0.235	0	0	0	0	7.326
	Wheat	12	6.898	0.162	0.044	0.276	0.006	0.012	0.010	0	7.408
	Wheat + E	12	7.048	0.154	0.055	0.262	0.003	0.010	0.007	0	7.539
	SE		0.7494	0.0608	0.0311	0.0404	0.0038	0.0081	0.0069		0.7290
	P		0.99	0.90	0.81	0.77	0.56	0.53	0.60		0.98
Caeca	Commercial	12	87.525	**6.520** [b]	11.099	0.211	0.508	0.366	0.011	0	106.241
	Wheat	12	102.137	**10.178** [a]	9.163	0.313	0.651	0.539	0	0	122.982
	Wheat + E	11	105.249	**5.816** [b]	17.932	0.180	0.666	0.298	0	0	130.141
	SE Com/Wheat		8.7511	1.3545	3.1335	0.0931	0.1985	0.1653	0.0064		10.0084
	SE Wheat + E		9.1402	1.4147	3.2729	0.0972	0.2073	0.1727			10.4534
	P		0.33	**0.04** [1]	0.15	0.58	0.83	0.58	0.40		0.25
Colon	Commercial	12	6.155	0.161	0.150	0.063	0	0.007	0.010	0	6.547
	Wheat	11	4.805	0.094	0.052	0.125	0	0	0.004	0	5.081
	Wheat + E	12	4.963	0.121	0.082	0.108	0.002	0.003	0.011	0	5.292
	SE Com/Wh + E		1.2756	0.0513	0.0538	0.0205	0.0012	0.0033	0.0068		1.2856
	SE Wheat		1.3323	0.0536	0.0562	0.0214			0.0071		1.3428
	P		0.72	0.67	0.44	0.11	0.40	0.40	0.76		0.69

[1] p = dry matter corrected. Values are original data.

Table 44. Influence of feeding a commercial crumbled grower or a cold-pelleted, crumbled wheat or wheat plus enzyme diet on the individual short chain fatty acid proportion (%) of total short chain fatty acid in the digesta in the distal ileum, caeca and colon of 15 week old growers.

Organ	Diet	N	Short chain fatty acid (C1-C7)							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Ileum	Commercial	12	0.944	0.021	0.005	0.030	0	0	0	0
	Wheat	12	0.924	0.029	0.007	0.036	0.001	0.002	0.002	0
	Wheat + E	12	0.926	0.025	0.009	0.036	0.001	0.002	0.001	0
	SE		0.0191	0.0105	0.0050	0.0055	0.0006	0.0013	0.0011	
	P		0.71	0.86	0.82	0.70	0.57	0.53	0.60	
Caeca	Commercial	12	0.818	0.074	**0.095** [ab]	0.003	0.005	0.006	0.0001	0
	Wheat	12	0.819	0.097	**0.068** [b]	0.005	0.005	0.008	0	0
	Wheat + E	11	0.815	0.044	**0.132** [a]	0.001	0.005	0.003	0	0
	SE Com/Wheat		0.0294	0.0180	0.0227	0.0019	0.0015	0.0035	0.00004	
	SE Wheat + E		0.0307	0.0188	0.0236	0.0020	0.0016	0.0036		
	P		0.99	0.15	**0.046** [1]	0.53	0.97	0.58	0.40	
Colon	Commercial	12	0.895	0.048	0.038	0.015	0	0.003	0.001	0
	Wheat	11	0.921	0.033	0.016	0.029	0	0	0.001	0
	Wheat + E	12	0.917	0.031	0.022	0.025	0.001	0.001	0.003	0
	SE Com/Wh + E		0.0323	0.0187	0.0119	0.0060	0.0005	0.0014	0.0013	
	SE Wheat		0.0338	0.0195	0.0124	0.0063			0.0013	
	P		0.84	0.79	0.42	0.26	0.40	0.36	0.39	

[1] p = probability derived from log transformed data. Data are original values.

8.4 Discussion

Several factors provided results that may be connected to indicate that an acidosis was present in the hindgut in birds fed the wheat diets and that the enzyme acted to moderate the effects. The excreta pH was reduced significantly when wheat-based feeds were introduced to the birds. The alteration over the 12 h periods of the experiment was contradictory to most of the earlier results with 24 and 48 h providing indications of substantial pH reduction. It was suggested earlier that at 12 and 36 h, the days digesta flow through the lower gut may have provided substrate to allow for expression of fermentative effects whilst at 24 and 48 h, at or slightly after a larger feed following the long dark period, little rapidly fermentable substrate would be present. Any metabolites of active fermentation may have been largely cleared at the time of measurement. In this experiment a different pattern was obvious. Given that other results indicate some fermentation (VFA and lactate results) this wheat may have been causing subtle negative effects. If a viscosity problem is reduced by enzyme application there was evidence that perhaps digesta flow was impeded and, hence, the pH reductions found in the excreta may be delayed. The pH of excreta from the enzyme supplemented birds was higher at 48 h than from the un-supplemented wheat diet.

The consistent decline in plasma pH was evident again and the wheat diets produced greater decline than the commercial diet. However, plasma lactate concentrations, though varying in time, provided little evidence of any unusual lactate accumulation. L-lactate concentration at 24 h decreased at the time when excreta pH declined which does not suggest any systemic acid effect.

Lactic acid concentrations in the digesta were another matter. Concentrations in the ileum and colon were greater than that observed in previous trials. In the caecal digesta, un-supplemented wheat-fed birds had lactic acid concentrations that would be indicative of an acidotic condition. The concentrations on the commercial diet were elevated as well and this could be explained by the commercial feed containing some of the same wheat used in the trial diets. The enzyme was reducing the effect in birds fed the wheat diet.

Digesta pH was little different across feeds and was similar to results from previous experiments other than for the caecal pH's which were low. Fermentative activity was evident in the VFA results with enhanced production in all the gut segments compared with previous results. The combination of the lactate and VFA figures indicates quite a deal more total acid being produced. Although the totals are a great deal lower than those reported by other workers (Carre *et al*., 1995; Choct *et al*., 1996; Williams *et al*., 1997), growers have a much lower feed intake which may reduce the potential for fermentation. Additionally, sampling for the current experiments was done within a short period of the morning feed and little digesta had progressed to the hindgut, so pre-empting any surge in activity. The different propionic acid concentrations across the feeds was novel but as the proportions differed little from the earlier work, no sensible interpretation could be proposed.

These results led to consideration of alterations in feed presentation methods which may affect the digestive process.

9. Effects of a commercial or wheat-based crumbled diet and altered feeding methods on gut pH and fermentation.

9.1 Introduction

Alternative feeding methods have been intensively researched over many years with the aim of production improvements. Recently, considerable attention has been devoted to wet-feeding whereby feed or grain is soaked in water prior to feeding. Yasar and Forbes (1997a; 1997b 1999) investigated gut effects, including digesta pH, using various diets and water treatments. An attempt to cover the range of effects of various dietary manipulations was made in a complex series of trials by Preston *et al*. (2000). In these trials results were at times confusing and concentrated on production effects. As well, broilers were the test bird and few recent trials have used layer types to monitor gut effects of altering feeding treatment methods.

The following experiment was performed to monitor digesta effects when growers were fed a commercial or full wheat-based ration *ad libitum*, as distinct morning and evening "meals" or after soaking in water.

9.2 Materials and methods

Group II birds, reared as per General Materials and Methods (above), were selected as laying birds a week after lay had commenced and were allocated to treatments (n=12) at 126 d old at lights on on d 1 after 5 d in the experimental cages. The experiment was a two-factor factorial design with birds allocated to continuing on the commercial ration (feed 1) or the wheat-based, cold-pelleted and crumbled ration (feed 2) as described in the previous experiment. Each feed was presented by one of three methods; 1. *ad libitum*; 2. "meal" feeding whereby birds were allowed access to feed for one hour at lights-on and for another hour prior to lights-off, and 3. "wet" feeding whereby the feed was mixed with an equivalent mass of hot (50ºC) water, allowed to cool for 10 min then approximately 300 g was placed in the individual feed troughs.

As described in the previous experiment, at slaughter, additional samples of ileal and caecal digesta were collected and dried for calculation of the SCFA content in the digesta on a DM basis.

9.3 Results

Feed intake was not measured due to difficulty associated with differential moisture loss in the remnants of the "wet" feed and interference to the birds associated with measuring the evening "meal" consumption. It was apparent that feed intake was similar on the two dry feeds; "meal" fed birds adjusted to the method by the second feed. "Wet" fed birds appeared to eat more and approached the feed with what could be described as enthusiasm.

Excreta pH (Table 45) was lower ($p < 0.05$) at 24 and 48 h than at 0, 12 and 36 h. Wheat diets reduced ($p < 0.05$) excreta pH irrespective of the method of feeding (7.37^{a} and $7.17^{b} \pm 0.039$ for commercial and wheat feeds respectively). The commercial diet produced similar ($p > 0.05$) pH across feed methods and the wheat diet that was meal fed; the wheat diets fed *ad libitum* and "wet" produced a lower ($p < 0.05$) pH than the other diets combinations other than for the meal fed commercial diet ($p > 0.05$) (7.40^{a}, 7.28^{ab}, 7.43^{a}, 7.11^{b}, 7.30^{a} and $7.11^{b} \pm 0.067$ for the commercial – *ad lib*., meal and wet and wheat – *ad lib*., meal and wet respectively). Excreta pH from 12 to 48 h was not affected ($p > 0.05$) by the excreta or plasma pH at time 0.

Table 45. Influence of commercial or wheat-based diets, fed by one of three methods, on the fresh excreta pH of layers (n=12) at 18/19 weeks old.

Factor	Time (h)				
	0	12	24	36	48
Mean excreta pH	7.47^{a}	7.42^{a}	7.05^{b}	7.50^{a}	6.92^{b}
SE			0.061		
Commercial	7.73	7.45	7.07	7.61	7.01

Wheat	7.22	7.40	7.03	7.39	6.83
SE			0.087		

Caecal evacuation pH (Table 46) was not significantly ($p > 0.05$) affected by feed type or the method of feeding over the trial. The lack of samples available, varying greatly at each collection, resulted in large standard errors.

Table 46. Influence of commercial or wheat-based diets, fed by one of three methods, on the fresh caecal evacuation pH of layers (n=12) at 18/19 weeks old.

Factor	Time (h)				
	0	12	24	36	48
Mean caecal digesta pH	6.91	6.70	6.68	6.81	6.62
SE	0.101	0.081	0.070	0.091	0.068
Commercial	7.13	6.72	6.60	6.82	6.62
Wheat	6.69	6.68	6.77	6.81	6.62
SE	0.141	0.164	0.139	0.178	0.136

Plasma pH decreased ($p < 0.05$) over time (Table 47) and a complex time * feed interaction ($p < 0.05$) was found whereby pH decreased from 0 to 24 h then was maintained on the commercial feed but did not fall significantly from 0 h until 48 h on the wheat diet. The method of feeding had no significant ($p > 0.05$) influence on plasma pH.

Table 47. Influence of commercial or wheat-based diets, fed by one of three methods, on the chilled plasma pH of layers (n=12) at 18/19 weeks old.

Feed	Time (h)		
	0	24	48
Mean plasma pH	7.748 [a]	7.712 [b]	7.692 [c]
SE	0.0070		0.0071
Commercial	7.754 [a]	7.702 [cd]	7.707 [c]
Wheat	7.742 [ab]	7.723 [bc]	7.677 [d]
SE	0.0099		0.0102

Feeding method did not alter ($p > 0.05$) gut section digesta or macerated kidney pH (Table 48) although that for the jejunum approached significance ($P = 0.0547$). The wheat-based diet changed digesta pH with an increase ($p < 0.05$) in the gizzard and caecum and decrease ($p < 0.05$) in the crop compared with the commercial diet. The remaining gut section digesta and macerated kidney pH's were unaffected ($p > 0.05$) by diet.

Table 48. Gut section digesta and kidney pH of birds euthanased after 48 h access to a commercial or wheat-based diet.

Gut section	Feed	LS Mean	SE (LS mean)	p
CROP	Commercial	5.08 [a]	0.066	**0.014**
	Wheat	4.84 [b]	0.067	
GIZZARD	Commercial	3.72 [b]	0.080	**0.001**
	Wheat	4.33 [a]	0.081	
JEJUNUM	Commercial	7.15	0.076	0.465
	Wheat	7.07	0.076	

ILEUM	Commercial	8.85	0.032	0.084
	Wheat	8.72	0.033	
CAECUM	Commercial	5.69 [b]	0.068	**0.001**
	Wheat	6.50 [a]	0.072	
COLON	Commercial	8.41	0.106	0.561
	Wheat	8.49	0.101	
KIDNEY	Commercial	6.807	0.0114	0.109
	Wheat	6.781	0.0116	

Plasma L- or D-lactic acid (Table 49) were not significantly ($p > 0.05$) altered by feed type or method. L-lactic acid decreased ($P < 0.05$) from 0 to 24 h then returned to the original level and at 48 h D-lactic acid levels were greater ($P < 0.05$) than at 0 and 24 h.

Table 49. Effects of commercial or wheat-based diets, fed by one of three methods, on the plasma concentration of L- and D-lactic acid (mMol/L) of layers (n=12) at 18/19 weeks old.

Factor	Time (h)					
	0	24	48	0	24	48
	L-lactic acid			D-lactic acid		
Plasma concentration (mMol/L)	5.89 [a]	5.22 [b]	5.81 [a]	0.021 [b]	0.010 [b]	0.038 [a]
SE	0.190	0.201	0.190	0.0046	0.0047	0.0046
Commercial	5.61	5.20	6.12	0.025	0.008	0.033
Wheat	6.17	5.24	5.51	0.017	0.012	0.043
SE	0.273	0.290	0.273	0.0065	0.0067	0.0065
ad libitum	5.84	5.16	5.98	0.028	0.005	0.031
Meal	5.64	5.07	5.68	0.013	0.008	0.033
Wet	6.20	5.44	5.78	0.022	0.017	0.051
SE	0.332	0.355	0.332	0.0080	0.0085	0.0080

Analysis - main effects		
Factor	P	P
Time	**0.0339**	**0.0001**
Feed	0.9766	0.7126
Method	0.4535	0.1414
All interactions	NS	NS

Digesta dry matter content (Table 51) was similar ($p > 0.05$) in both the ileum and caeca (0.791 and 0.778 ± 0.0054 respectively) and was not affected ($p > 0.05$) by feed type or method in the ileum. However, in the caeca "wet" feeding produced a wetter ($P < 0.05$) excreta than meal feeding with standard *ad libitum* feeding similar ($p > 0.05$) to both other methods (0.776 and 0.807 ± 0.0119 and 0.753 ± 0.0122 for *ad lib*., meal and wet feeding respectively).

Table 51. Digesta dry matter (%) in the ileum and caeca of 18/19 week old layers euthanased after 48 h access to a commercial or wheat-based diet fed by one of three methods.

Gut section	Feed	Method	LS Mean	SE (LS mean)
ILEUM	Commercial	*Ad libitum*	0.789	0.0081
		Meal	0.784	0.0081
		Wet	0.782	0.0081

	Wheat	*Ad libitum*	0.807	0.0081
		Meal	0.800	0.0081
		Wet	0.785	0.0084
CAECUM	Commercial	*Ad libitum*	0.766	0.0169
		Meal	0.785	0.0169
		Wet	0.765	0.0169
	Wheat	*Ad libitum*	0.787	0.0169
		Meal	0.828	0.0169
		Wet	0.741	0.0176

Short chain fatty acid concentration (mMol/L) (Table 52) in the ileum was greater ($p < 0.05$) for acetic and total acid in the birds fed the commercial diet than those fed the wheat-based diet. Heptanoic acid concentration was greater ($p < 0.05$) in birds fed the commercial diet ad libitum than those that were meal or wet-fed which produced similar ($p > 0.05$) concentrations and the wheat-fed birds had a lower ($p < 0.05$) concentration than those fed the commercial diet. Digesta dry matter correction did not alter ($p > 0.05$) SCFA concentrations across treatments in the ileum. The proportion of total SCFA (Table 53) in the ileum was lower ($p < 0.05$) for iso-butyric and greater ($p < 0.05$) for heptanoic acids in birds fed the commercial rather than the wheat diet. The method of feeding had no effect ($p > 0.05$) on SCFA proportions.

In caecal digesta, dry matter correction resulted in changes to several significant results; differences in acetic and iso-butyric acid concentrations were rendered non-significant ($p > 0.05$). Both n- and iso-valeric acids were in greater concentration in birds fed the wheat-based diet. The proportion of propionic, n- and iso-valeric acids of total SCFA was greater ($p < 0.05$) in wheat-fed birds and log-transformed data resulted indicated a greater ($p < 0.05$) iso-butyric acid proportion in wheat-fed birds. Feeding method did not influence ($p > 0.05$) either SCFA concentration or proportion.

In the colon, more ($p < 0.05$) iso-butyric and less ($p < 0.05$) heptanoic acid concentration and proportion of total SCFA was found in birds fed the wheat-based diet.

Table 52. Influence of feeding a commercial, crumbled or wheat-based cold-pelleted, crumbled diet on the concentration (mMol/L) of short chain fatty acid (C1-C7) in digesta content in the distal ileum, caeca and colon of 18/19 week old female layers.

Organ	Diet	Method	N	Short chain fatty acid (C1-C7)								
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	Total
Ileum	Commercial	*ad lib*.	12	**4.776** [a]	0.079	0.033	0.269	0.022	0.020	0.084	**0.140** [a]	**5.423** [a]
		meal	12	**5.019** [a]	0.040	0.028	0.233	0.006	0.006	0.078	**0.111** [b]	**5.523** [a]
		wet	12	**4.803** [a]	0.015	0	0.232	0	0	0.079	**0.113** [b]	**5.243** [a]
	Wheat	*ad lib*.	12	**1.862** [b]	0	0	0.190	0	0.001	0.033	**0.005** [c]	**2.090** [b]
		meal	12	**1.651** [b]	0	0	0.209	0	0	0.033	**0.009** [c]	**1.902** [b]
		wet	10	**2.216** [b]	0	0	0.242	0	0.001	0.062	**0.008** [c]	**2.528** [b]
		SE		0.5631	0.0248	0.0159	0.0239	0.0093	0.0085	0.0209	0.0073	0.5880
		SE W/wet		0.6168			0.0261		0.0094	0.0229	0.0080	0.6441
		P		**0.01**	0.17	0.44	0.27	0.51	0.55	0.30	**0.01**	**0.01**
Caeca	Commercial	*ad lib*.	12	71.067	4.431	12.681	0.154	**0.355** [b]	**0.059** [a]	0.012	0.019	88.775
		meal	12	90.828	6.696	15.374	0.122	**0.311** [b]	**0.017** [a]	0.005	0.014	113.367
		wet	12	86.646	5.908	17.143	0.188	**0.288** [b]	**0.020** [a]	0	0.017	110.208
	Wheat	*ad lib*.	12	72.105	6.839	14.024	0.158	**1.331** [a]	**0.198** [b]	0.016	0.012	94.685
		meal	11	64.102	5.839	10.839	0.138	**0.897** [a]	**0.188** [b]	0.008	0.007	82.017
		wet	11	71.916	7.624	13.989	0.325	**1.402** [a]	**0.351** [b]	0.012	0.008	95.627
		SE		5.8609	0.7628	2.4557	0.0365	0.1647	0.0358	0.0102	0.0094	8.1347
		SE W/m w		6.1215	0.7967	2.5648	0.0381	0.1720	0.0374	0.0107	0.0098	8.4964
		P		0.24[1]	0.21	0.69	0.30[1]	**0.01**[1]	**0.0497**[1]	0.92	0.94	0.31
Colon	Commercial	*ad lib*.		10.724	0.123	0.296	**0.071** [b]	0.019	0.022	0.040	**0.051** [a]	11.344
		meal		13.095	0.163	0.738	**0.045** [b]	0	0.001	0.011	**0.030** [a]	14.083
		wet		9.511	0	0.018	**0.049** [b]	0	0	0.017	**0.033** [a]	9.627
	Wheat	*ad lib*.		10.867	0.002	0.036	**0.543** [a]	0.031	0.006	0.023	**0.004** [b]	11.512
		meal		10.671	0.012	0.013	**0.563** [a]	0.021	0	0.022	**0.008** [b]	11.309
		wet		12.886	0.146	0.327	**0.550** [a]	0.060	0.006	0.025	**0.005** [b]	14.006
		SE		2.2709	0.0934	0.3258	0.0516	0.0169	0.0090	0.0143	0.0078	2.655
		P		0.81	0.56	0.48	**0.01**	0.09	0.49	0.77	**0.01**	0.77

[1] p = dry matter corrected. Values are original data.

Table 53. Influence of feeding a commercial, crumbled or wheat-based cold-pelleted, crumbled diet on the individual short chain fatty acid proportion (%) of SCFA in the digesta content in the distal ileum, caeca and colon of 18/19 week old layers.

Organ	Diet	Feed	N	Short chain fatty acid (C1-C7)							
				acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Ileum	Commercial	*ad lib*.	12	0.881	0.013	0.006	**0.051** [b]	0.004	0.003	0.016	**0.027** [a]
		meal	12	0.897	0.006	0.004	**0.050** [b]	0.001	0.001	0.015	**0.025** [a]
		wet	12	0.898	0.003	0	**0.054** [b]	0	0	0.019	**0.026** [a]
	Wheat	*ad lib*.	12	0.851	0	0	**0.137** [a]	0	0.001	0.011	**0.001** [b]
		meal	12	0.859	0	0	**0.124** [a]	0	0	0.014	**0.003** [b]
		wet	10	0.859	0	0	**0.115** [a]	0	0.001	0.023	**0.002** [b]
		SE		0.0201	0.0043	0.0026	0.0154	0.0016	0.0015	0.0063	0.0023
		SE W/wet		0.0220			0.0168		0.0017	0.0069	0.0025
		P		0.41	0.24	0.46	**0.01**	0.52	0.64	0.85	**0.01**
Caeca	Commercial	*ad lib*.	12	0.806	**0.052** [b]	0.133	**0.002** [b]	**0.004** [b]	**0.0015** [b]	0.0004	0.0004
		meal	12	0.808	**0.060** [b]	0.128	**0.001** [b]	**0.003** [b]	**0.0002** [b]	0.0001	0.0001
		wet	12	0.791	**0.053** [b]	0.151	**0.002** [b]	**0.002** [b]	**0.0002** [b]	0	0.0002
	Wheat	*ad lib*.	12	0.773	**0.073** [a]	0.133	**0.003** [a]	**0.014** [a]	**0.0030** [a]	0.0007	0.0005
		meal	11	0.791	**0.079** [a]	0.113	**0.002** [a]	**0.010** [a]	**0.0035** [a]	0.0001	0.0001
		wet	11	0.757	**0.077** [a]	0.143	**0.003** [a]	**0.014** [a]	**0.0041** [a]	0.0001	0.0001
		SE		0.0209	0.0066	0.0197	0.0006	0.0016	0.00087	0.00033	0.00025
		SE W/m w		0.0218	0.0069	0.0206	0.0007	0.0017	0.00091	0.00035	0.00026
		P		0.53	**0.01**	0.84	**0.01**[1]	**0.01**	**0.01**	0.69	0.75
Colon	Commercial	*ad lib*.		0.941	0.013	0.018	**0.009** [b]	0.003	0.00364	0.006	**0.0061** [a]
		Meal		0.968	0.005	0.019	**0.004** [b]	0	0.0001	0.001	**0.0030** [a]
		Wet		0.989	0	0.002	**0.005** [b]	0	0	0.002	**0.0033** [a]
	Wheat	*ad lib*.		0.946	0.001	0.003	**0.046** [a]	0.002	0.0004	0.002	**0.0003** [b]
		Meal		0.947	0.001	0.001	**0.047** [a]	0.002	0	0.002	**0.0012** [b]
		Wet		0.939	0.003	0.008	**0.044** [a]	0.003	0.0005	0.002	**0.0005** [b]
		SE		0.0163	0.0045	0.0073	0.0045	0.0013	0.00139	0.0020	0.00116
		P		0.16	0.28	0.24	**0.01**	0.34	0.39	0.65	**0.01**

[1] p = probability derived from log transformed data. Data are original values of LS means

9.4 Discussion

Similar patterns of changes in excreta pH were found in this, as in the last experiment. The wheat diets utilised the same grains used in previous experiments but the commercial diet, sourced from another miller was of unknown composition. Caecal evacuations provided little evidence of any substantial shift in pH. This experiment allowed one of the few opportunities for consistent caecal content collection and the effort expended in attempting sensible measures over the course of the project suggests that it is not a viable option for monitoring changes in digesta.

Plasma pH changes were, again, consistent in falling over the trial period. There was a fall in pH which was different across feeds in that a great reduction was delayed in the wheat-fed birds. Perhaps the previously propounded idea that this wheat was causing some greater digesta viscosity and, thus, slowing digestion and, hence, responses to the diet change, was valid.

One curiosity was the low caecal pH produced by the commercial diet compared with that of the wheat-fed birds. Possibly, the "reduced digesta flow rate" suggestion may have had an effect or the activities of the microbial populations were affected by the wheat diet.

Plasma lactate concentrations were remarkably similar to the previous trial results and feeding methods had little effect. With the rapid adjustment of the birds to both a wet mash and "meal" feeding it seems that intake was unaffected and no untoward experimental bias was caused. Whether these methods of presenting the feed would have an effect over a period in excess of 48 h would be worth pursuing.

Financial constraints precluded lactate measurement in the digesta; another area for further work given results from the enzyme trial conducted with the same grain. This is highlighted by the substantial VFA concentrations found in the ileum and colon as in the previous trial. The production of the C6 and C7 acids was of interest as little was found in lower gut sections in the earlier work. Feed differences in the *iso*- forms (valeric in the caeca and butyric in the colon) were of note. The sources of the protein being fermented in the different gut sections requires elucidation. These VFA's are usually only produced in minute quantity but with up to 10 x the product being due to a feed change, leads to speculation as to whether endogenous, feed or microbial protein is the source.

10. Additional broiler trials

10.1 Introduction

Several methodologies such as pH measurement of fresh excreta, fresh caecal evacuations and digesta samples required trial for the contracted layer bird experiments. Further, manipulations of cereal processing in conjunction with effects of commercial exogenous feed enzymes were of interest following an introductory experiment with triticale diets where the triticale was included in a sorghum-basal diet. The triticale experiment highlighted some production responses in broilers whereby a poorer feed conversion efficiency in the starter phase with 200 g/kg whole grain inclusion in the mix prior to pelleting was reversed in the grower phase and a compensatory improvement in response resulted in equal performance in whole-grain treated feeds to those that were enzyme supplemented.

A follow-up experiment was conducted using wheat, again in a sorghum-basal diet, and results were somewhat equivocal. In both experiments, responses in gut physiology were prominent and a difference in ascites losses with cereal grain processing were noted.

These experiments were reported in conference proceedings (see Compendium Summary below) and are not included in the current report. As wheat and barley are of interest to poultry production, a full-wheat diet was deemed necessary for further study of gut responses as was barley inclusion in the aforementioned sorghum basal diet and the following two experiments were conducted to introduce methods and pursue hypotheses tangential to the project aims.

10.2 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 eight to the brooders. At 18 d of age, the groups of birds were transferred to large wire-mesh cages where they remained until 42 d of age.

The experiments were both of a 2 x 2 factorial, randomised within-blocks design (Snedecor and Cochran, 1980) replicated eight times.

Experiment 1
The birds were fed one of two commercially formulated dietary treatments with or without the inclusion of an exogenous food enzyme (Allzyme PT (endo-1,4, ß-xylanase EC 3.2.1.8, 1000 XU/g), Alltech; 1.0 g/kg diet) and which were offered as a starter food (12.10 MJ ME/kg, 214 g CP/kg) from 5-21 d of age and as a grower food (12.26 MJ ME/kg, 217 g CP/kg) from 22-42 d of age (Table 54).

The dietary treatments were identical except that 200 g/kg of the wheat (a 60:40 Durum:bread wheat blend of old season grain supplied by Weston Animal Nutrition, Tamworth, NSW) was included in the mix as either whole or hammermilled (6 mm diameter screen) grain.

Experiment 2
The birds were fed one of two commercially formulated dietary treatments with or without the inclusion of an exogenous food enzyme (Allzyme BG (ß-glucanase EC 3.2.1.6, 650 BGU/g), Alltech; 1.0 g/kg diet) and which were offered as a starter food (12.10 MJ ME/kg, 215 g CP/kg) from 5-21 d of age and as a grower food (12.30 MJ ME/kg, 213 g CP/kg) from 22-42 d of age (Table 54). The dietary treatments were similar to those used in Experiment 1, except that whole or finely

hammermilled barley (2-row type mix of malting varieties which had failed malting classification supplied by Weston Animal Nutrition, Tamworth, NSW) was included at 200 g/kg on a finely hammermilled sorghum basal diet.

After mixing, the diets were cold pelleted, 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.

The birds were weighed at 21 and 42 d of age. Food conversion ratio (FCR; g food intake/g bodyweight gain) was determined for the starter (5-21 d) and grower (22-42 d) phases as well as between 5 and 42 d. Birds showing an inability to move around the cage and to obtain food and water, due to leg abnormalities, were culled. All bird mortalities and their causes, throughout the course of the experiment, were recorded.

Table 54. Experimental broiler starter and grower diets (g/kg).

Raw	Experiment 1		Experiment 2	
	Starter	Grower	Starter	Grower
Wheat (120 g/kg CP)	675.5	665.1		
Sorghum (90g/kg CP)			448.8	442.8
Barley (100g/kg CP)			200.0	200.0
Soybean meal (475 g/kg CP)	187.2	208.8	244.4	245.3
Meat meal (520 g/kg CP)	69.7	65.7	67.8	67.9
Millrun (160 g/kg CP)	11.4			
Tallow	30.7	36.7	20.9	28.3
Vegetable oil	5.0	5.5	2.2	2.2
Limestone	0.9	1.9	1.1	1.1
Potassium carbonate	3.5	2.8		
Lysine HCl	3.2	1.3	2.1	0.5
DL-Methionine	2.9	3.2	3.3	3.3
L-Threonine	0.5		0.1	
Salt	0.8	1.3	0.9	1.4
Sodium bicarbonate	3.5	2.3	3.2	2.0
Choline chloride	0.3	0.5	0.3	0.3
[1]Vitamin/mineral premix	5.0	5.0	5.0	5.0
Calculated specifications (% unless otherwise specified)				
Metabolisable energy (MJ ME/kg)	12.10	12.26	12.10	12.30
Crude Protein (g/kg)	214	217	215	213
Lysine (g/kg)	12.5	11.5	12.5	11.3
Methionine + Cystine (g/kg)	9.1	9.5	9.1	9.1

[1] The active ingredients (mg/kg) contained in the vitamin and mineral premix were as follows: retinol 3; cholecalciferol 0.075; all-*rac*-α-tocopheol acetate 80; menadione 3; riboflavin 5; pyridoxine HCl 2.97; biotin 0.12; niacin 20; thiamine 1.98; D-calcium pantothenate 8.01; folic acid 1.5; antioxidant 125; Mn 100; Fe 50; Cu 15.4; I 1.5; Se 0.15; Mo 0.99; cyanocobalamin 0.025.

Excreta pH was measured on fresh samples collected from under each cage at irregular intervals after the starter and grower feeds were introduced to the birds. The excreta trays were scraped clean and excreta from 4 different fresh droppings were collected in specimen tubes. Care was taken to exclude excreta contaminated with either caecal evacuations or uric acid. The excreta pH was measured as described in General Materials and Methods (above).

Mid-week for each week from 10 days of age (i.e. on trial feed for 5 d) a 24 h total excreta collection from each cage was made for the determination of moisture content.

At 42 d of age, three randomly selected birds from each group were slaughtered by cervical dislocation and dissected to determine organ responses to the diets. The proventriculus was scored for the presence or absence of dilatation (binary data; 1 = dilatation, 0 = no dilatation). The proventriculus and gizzard were removed and full and empty fresh weights determined. The full and empty weights and empty lengths of the duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to 1 cm above ileocaecal junction) were recorded. Samples of digesta from the crop and the distal ends of the jejunum, ileum and caeca of each of the three birds were squeezed gently into 4 ml tubes and stored on ice. The digesta, or remaining digesta, was expressed into specimen tubes and the pH measured after treatment as per excreta samples.

Digesta samples were treated in two ways. Samples from two of the sacrificed birds per cage were centrifuged at 10,000 G for 20 min, the supernatant was transferred to 1.5 ml microfuge tubes which were stored at -20 ° C. After thawing to 20 °C in an air-conditioned room, maintained at that temperature, supernatant viscosity was measured on a Brookfield DV II viscometer. The remaining samples were prepared for SCFA measures as described in General Materials and Methods (above).

The broiler trial production data and some gut measures were analysed by analysis of variance using Genstat 5 (Release 3.1, Lawes Agricultural Trust, Rothamsted, U.K.) to report Standard Errors of Differences of means. Other data employed the GLM and MIXED Model procedures of SAS as above. Probabilities in tables are denoted as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

10.3 Results

Results of body weight gain, feed conversion efficiencies, gut organ measurements including counts of proventricular dilatation and ascites mortalities have been presented in two conference proceedings and one journal publication (see Compendium Summary).

Digesta content in the ileum of wheat-fed birds (Table 55) was greater ($p < 0.05$) on the ground diet without enzyme supplementation than on the other diets and in the caeca when fed the same diet although in the caeca the enzyme supplemented, whole-grain inclusion diet had a greater ($p < 0.05$) content again. On the barley diet, digesta content (Table 56) was greater ($p < 0.05$) in the gizzard and lower ($p < 0.05$) in the jejunum of birds fed the whole-grain inclusion diet rather than the ground diets. There was no effect ($P > 0.05$) of enzyme addition. Digesta contents were similar ($p > 0.05$) in the other gut organs on both diets. Digesta pH (Tables 57 and 58) was largely unaltered ($p > 0.05$) by either grain with feed processing or enzyme addition other than for duodenal pH being ($p < 0.05$) reduced on the barley diet fed as fully ground and with enzyme addition. Digesta viscosities (Tables 57 and 58) were low throughout the digestive tract irrespective of the cereal fed. Whole-grain inclusion resulted in higher ($p < 0.05$) gut viscosity in the crop, jejunum and ileum of wheat-fed birds and the duodenum of barley-fed birds. Enzyme inclusion reduced ($p < 0.05$) viscosity in the duodenum, jejunum and ileum of the wheat-fed birds.

Excreta moisture (Table 59) content increased from the first week and alternated between 73 and 77% over the final three weeks irrespective of the cereal. For the first 18 d the birds were in the brooders and the decreasing heat levels would affect excreta drying. There was a greater ($p < 0.05$) excreta moisture content in the wheat-fed birds given the enzyme supplement.

Table 55. Influence of feeding diets containing ground or whole wheat and with or without exogenous enzyme addition on the weight (g/kg bodyweight (BW)) of digesta content in the gizzard and intestinal segments and full caeca of 42-d-old male broiler chickens.

Diet	Enzyme[1]	Gizzard (g/kg BW)	Duodenum (g/kg BW)	Jejunum (g/kg BW)	Ileum (g/kg BW)	Caeca (full) (g/kg BW)
Ground	–	1.4	1.9	8.8	7.4	7.5
	+	0.8	2.0	8.0	4.8	6.0
Whole	–	2.0	1.5	7.2	5.0	6.1
	+	2.6	1.5	7.0	5.1	8.7
SE	Form (F)	0.36	0.12	0.43	0.35*	0.32
	Enzyme(E)	0.36	0.12	0.43	0.35*	0.32
	F x E	0.51	0.17	0.61	0.49**	0.45***

[1]Allzyme PT (1.0 mg/kg)

Table 56. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the weight (g/kg bodyweight (BW)) of digesta content in the gizzard and intestinal segments and full caeca of 42-d-old male broiler chickens.

Diet	Enzyme[1]	Gizzard (g/kg BW)	Duodenum (g/kg BW)	Jejunum (g/kg BW)	Ileum (g/kg BW)	Caeca (full) (g/kg BW)
Ground	–	4.4	1.2	9.2	7.8	6.4
	+	3.6	1.3	10.4	8.4	5.9
Whole	–	8.0	1.0	8.0	7.9	6.2
	+	7.9	1.0	8.0	7.6	6.0
SE	Form (F)	0.41***	0.08	0.37***	0.29	0.20
	Enzyme(E)	0.41	0.08	0.37	0.29	0.20
	F x E	0.57	0.11	0.52	0.42	0.28

[1]Allzyme BG (1.0 mg/kg)

Table 57. Influence of feeding diets containing ground or whole wheat and with or without exogenous enzyme addition on the pH and viscosity (cP) of digesta content in the crop and intestinal segments of 42-d-old male broiler chickens.

Diet	Enzyme[1]	Digesta pH				Digesta viscosity (cP)			
		Crop	Duodenum	Jejunum	Ileum	Crop	Duodenum	Jejunum	Ileum
Ground	–	4.46	5.87	6.21	6.79	1.42	2.00	2.39	4.25
	+	4.71	5.75	6.16	6.99	1.49	1.72	2.22	3.78
Whole	–	5.06	5.91	6.25	7.20	1.91	1.96	2.91	5.48
	+	4.93	5.89	6.24	6.96	1.79	1.91	2.38	4.15
SE	Form (F)	0.129	0.034	0.026	0.099	0.103**	0.050	0.070***	0.198**
	Enzyme(E)	0.128	0.034	0.026	0.099	0.103	0.048*	0.070***	0.196**
	F x E	0.192	0.049	0.037	0.144	0.153	0.072	0.100	0.290

[1]Allzyme PT (1.0 mg/kg)

Table 58. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the pH and viscosity (cP) of digesta content in the crop and intestinal segments of 42-d-old male broiler chickens.

Diet	Enzyme[1]	Digesta pH				Digesta viscosity (cP)			
		Crop	Duodenum	Jejunum	Ileum	Crop	Duodenum	Jejunum	Ileum
Ground	–	5.33	6.05	6.35	7.10	2.12	1.95	2.56	3.04
	+	5.14	5.89	6.30	7.09	1.90	1.86	2.40	3.09
Whole	–	5.64	5.99	6.36	7.65	2.04	2.01	2.71	3.11
	+	5.38	6.03	6.50	7.51	2.10	2.13	2.77	3.21
SE	Form (F)	0.092	0.029	0.056	0.167	0.077	0.049*	0.078	0.114
	Enzyme(E)	0.095	0.029	0.056	0.167	0.080	0.048	0.078	0.114
	F x E	0.137	0.042*	0.080	0.239	0.113	0.072	0.108	0.163

[1]Allzyme BG (1.0 mg/kg)

Table 59. Influence of feeding diets containing ground or whole wheat or barley and with or without exogenous enzyme addition on the water content (%) of excreta of 42-d-old male broiler chickens.

Diet	Enzyme[1,2]	Age (d)									
		Wheat/Wheat diet					Barley/Sorghum basal diet				
		10	17	24	31	38	10	17	24	31	38
Ground	–	62.1	71.9	74.9	72.8	76.0	59.3	68.0	75.5	78.1	74.1
	+	62.2	72.3	77.5	74.7	77.2	58.2	69.6	74.7	77.6	73.8
Whole	–	62.5	73.2	75.9	72.8	75.5	58.8	69.6	75.2	78.1	73.7
	+	63.6	73.5	77.6	75.4	77.1	58.1	68.8	75.0	77.7	72.4
SE	Form (F)			0.66					0.57		
	Enzyme(E)			0.66					0.57		
	F x E			0.93					0.81		
Analysis of Main Effects											
Factor	Wheat	Barley									
Time	0.0001	0.0001	62.6 [a]	72.7 [b]	76.5 [c]	73.9 [b]	76.4 [c]	58.6 [a]	69.0 [b]	75.1 [d]	77.9 [e]	73.5 [c]
					0.46					0.41		
Feed	0.1854	0.6082		72.2		72.7			70.9		70.7	
					0.29					0.26		
Enz	0.0015	0.2179		71.8 [a]		73.1 [b]			71.0		70.6	
					0.29					0.26		

[1]Allzyme PT (1.0 mg/kg) - wheat/wheat diet.
[2]Allzyme BG (1.0 mg/kg) - barley/sorghum diet.

Table 60. Influence of feeding diets containing ground or whole wheat and with or without exogenous enzyme addition on the excreta pH of male broiler chickens in the starter phase (1-21 d).

Diet	Enzyme[1]	Age (d)												
		1	2	3	4	5	6	7	8	10	11	12	14	19
Ground	–	7.08	6.74	6.10	6.05	6.60	6.81	7.01	7.31	7.54	7.59	7.28	7.19	7.20
	+	7.06	6.61	6.20	6.13	6.44	6.78	6.93	7.15	7.35	7.33	7.39	7.20	7.46
Whole	–	6.96	6.39	5.99	5.85	6.49	6.98	7.16	7.13	7.46	7.30	7.45	7.30	7.64
	+	6.93	6.50	5.96	6.18	6.21	6.66	7.13	6.83	7.28	7.35	7.64	7.44	7.48
						Start diet								
SE	Form (F)							0.086						
	Enzyme(E)							0.086						
	F x E							0.122						
Analysis of Main Effects														
Factor	P													
Time	0.0001	7.01[b]	6.56[d]	6.06[e]	6.05[e]	6.43[d]	6.81[c]	7.06[b]	7.10[b]	7.41[a]	7.39[a]	7.44[a]	7.28[a]	7.44[a]
								0.061						
Grain	0.3399				6.94						6.91			
								0.024						
Enz	0.2488				6.95						6.91			
								0.024						

[1]Allzyme PT (1.0 mg/kg)

Table 61. Influence of feeding diets containing ground or whole wheat and with or without exogenous enzyme addition on the excreta pH of male broiler chickens in the grower phase (22-42 d).

Diet	Enzyme[1]	Age (d)						
		22	25	27	29	32	35	38
Ground	–	6.26 [b]	6.35	6.49	5.48	6.51 [a]	5.74 [b]	6.31
	+	7.03 [a]	6.31	6.35	5.66	6.14 [b]	6.03 [b]	6.24
Whole	–	6.85 [b]	6.70	6.90	6.04	6.70 [a]	6.13 [b]	6.50
	+	7.14 [a]	7.11	6.84	6.41	6.48 [a]	6.69 [a]	6.51
		Start diet						
SE	Form (F)				0.103			
	Enzyme(E)				0.103			
	F x E				0.146			
Analysis of Main Effects								
Factor	P							
Time	0.0001	6.82 [a]	6.62 [abc]	6.64 [ab]	5.90 [e]	6.46 [bc]	6.14 [d]	6.42 [c]
					0.073			
Grain	0.0001		6.21 [b]				6.65 [a]	
					0.039			
Enz	0.0157		6.36 [b]				6.49 [a]	
					0.039			

[1]Allzyme PT (1.0 mg/kg)

Table 62. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the excreta pH of male broiler chickens in the starter phase (1-21 d).

Diet	Enzyme[1]	Age (d)						
		5	6	7	8	11	15	18
Ground	–		7.00	7.11	6.90 [a]	7.24	7.29	7.46 [a]
	+		6.79	7.21	7.15 [a]	7.40	7.14	7.36 [a]
Whole	–		6.59	6.93	6.44 [b]	7.36	7.16	6.91 [b]
	+		6.71	6.99	6.65 [b]	7.45	7.36	7.13 [b]
		Start diet						
SE	Form (F)				0.096			
	Enzyme(E)				0.096			
	F x E				0.135			
Analysis of Main Effects								
Factor	P							
Time	0.0001		6.77 [a]	7.06 [b]	6.78 [a]	7.36 [c]	7.24 [bc]	7.22 [bc]
					0.068			
Grain	0.0005		7.17 [a]				6.97 [b]	
					0.039			
Enz	0.1542		7.03				7.11	
					0.039			

[1]Allzyme BG (1.0 mg/kg)

Table 63. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the excreta pH of male broiler chickens in the grower phase (22-42 d).

Diet	Enzyme[1]	Age (d)									
		22	23	24	25	26	30	32	35	40	41
Ground	–		6.01 ^{b}	7.06 ^{ab}	7.20	7.24 ^{bc}	6.39 ^{b}	7.09 ^{b}	6.61 ^{b}	6.44 ^{b}	6.50 ^{b}
	+		6.31 ^{b}	6.91 ^{bc}	7.49	7.04 ^{c}	6.70 ^{b}	7.48 ^{ab}	6.98 ^{b}	6.41 ^{b}	6.90 ^{ab}
Whole	–		6.38 ^{b}	6.49 ^{c}	7.59	7.63 ^{ab}	6.66 ^{b}	7.96 ^{a}	7.49 ^{a}	7.06 ^{a}	7.03 ^{a}
	+		6.63 ^{a}	7.49 ^{a}	7.58	7.81 ^{a}	7.50 ^{a}	7.70 ^{a}	7.91 ^{a}	7.21 ^{a}	7.33 ^{a}
		Start diet									
SE	Form (F)					0.108					
	Enzyme(E)					0.108					
	F x E					0.153					
Analysis of Main Effects											
Factor	P										
Time	0.0001		6.33 ^{a}	6.99 ^{b}	7.46 ^{d}	7.43 ^{cd}	6.81 ^{b}	7.56 ^{d}	7.25 ^{c}	6.78 ^{b}	6.94 ^{b}
						0.076					
Grain	0.0001			6.82 ^{b}					7.30 ^{a}		
						0.036					
Enz	0.0001			6.93 ^{b}					7.19 ^{a}		
						0.036					

[1]Allzyme BG (1.0 mg/kg)

Table 64. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the pH of fresh caecal evacuations of male broiler chickens in the grower phase (22-42 d).

Diet	Enzyme[1]	Age (d)									
		22	23	24	25	26	30	32	35	40	41
Ground	–		6.56	6.40	6.10	6.54	6.66	6.20	6.19 [b]	6.46 [b]	6.59
	+		6.56	6.55	6.31	6.46	6.28	6.11	6.14 [b]	6.38 [b]	6.61
Whole	–		6.64	6.49	6.38	6.81	6.51	6.24	6.61 [a]	6.80 [a]	6.80
	+		6.48	6.55	6.14	6.58	6.46	6.25	6.67 [a]	6.63 [a]	6.64
		Start diet									
SE	Form (F)						0.087				
	Enzyme(E)						0.087				
	F x E						0.136				
Analysis of Main Effects											
Factor	P										
Time	0.0001		6.56 [ab]	6.50 [ab]	6.23 [c]	6.60 [a]	6.48 [ab]	6.20 [c]	6.40 [b]	6.57 [ab]	6.66 [a]
							0.058				
Grain	0.0001			6.39 [b]					6.54 [a]		
							0.026				
Enz	0.0734			6.50					6.43		
							0.026				

[1]Allzyme BG (1.0 mg/kg)

Table 65. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the short chain fatty acid (C1-C7) concentration (mMol/L) of digesta content in the distal ileum and caeca of 42-d-old male broiler chickens.

Organ	Diet	Enzyme[1]	Short chain fatty acid								
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic	total
Ileum	Ground	–	2.748	0.061	0.016	0.074	0.040	0.005	0.021	0.279	3.245
		+	2.861	0.049	0.004	0.112	0.015	0.001	0.004	0.180	3.227
	Whole	–	2.275	0.041	0.021	0.083	0.026	0.005	0.011	0.180	2.644
		+	3.072	0.043	0.008	0.096	0.014	0	0.003	0.207	3.444
	SE	Form (F)	0.2914	0.0128	0.0070	0.0276	0.0079	0.0025	0.0054	0.0365	0.2933
		Enzyme(E)	0.2914	0.0128	0.0070	0.0276	0.0079	0.0025	0.0054	0.0365	0.2933
		F x E	0.4121	0.0181	0.0099	0.0391	0.0112	0.0036	0.0076	0.0515	0.4147
Caeca	Ground	–	29.792	2.532	6.377	0.296	0.713	0.366	0	0	40.075
		+	41.304	3.334	9.394	0.278	0.921	0.350	0	0	55.580
	Whole	–	34.107	2.478	10.392	0.192	0.841	0.211	0	0	48.220
		+	42.250	3.080	12.071	0.099	0.934	0.152	0	0	58.586
	SE	Form (F)	4.4442	0.3899	1.3749	0.0655	0.1330	0.0827	0	0	6.1372
		Enzyme(E)	4.4442	0.3899	1.3749	0.0655	0.1330	0.0827	0	0	6.1372
		F x E	6.5645	0.5760	2.0308	0.0967	0.1965	0.1222	0	0	9.0653

[1]Allzyme BG (1.0 mg/kg)

Table 66. Influence of feeding diets containing ground or whole barley and with or without exogenous enzyme addition on the individual short chain fatty acid proportion (%) of total short chain fatty acid (C1-C7) in the digesta content in the distal ileum and caeca of 42-d-old male broiler chickens.

Organ	Diet	Enzyme[1]	Short chain fatty acid							
			acetic	propionic	*n*-butyric	*iso*-butyric	*n*-valeric	*iso*-valeric	hexanoic	heptanoic
Ileum	Ground	–	0.843	0.019	0.006	0.020	0.010	0.0020	0.002	0.098
		+	0.885	0.015	0.001	0.037	0.003	0.0001	0.001	0.060
	Whole	–	0.866	0.008	0.004	0.029	0.008	0.0030	0.004	0.078
		+	0.859	0.009	0.003	0.040	0.003	0	0.001	0.085
	SE	Form (F)	0.0194	0.0036	0.0023	0.0111	0.0028	0.0012	0.0013	0.0192
		Enzyme(E)	0.0194	0.0036	0.0023	0.0111	0.0028	0.0012	0.0013	0.0192
		F x E	0.0274	0.0051	0.0032	0.0158	0.0040	0.0017	0.0018	0.0271
Caeca	Ground	–	0.753	0.056	0.161	0.007	0.012	0.010	0	0
		+	0.783	0.063	0.131	0.005	0.012	0.007	0	0
	Whole	–	0.753	0.054	0.170	0.004	0.015	0.004	0	0
		+	0.789	0.060	0.136	0.002	0.012	0.002	0	0
	SE	Form (F)	0.0267	0.0066	0.0242	0.0014	0.0029	0.0019	0	0
		Enzyme(E)	0.0267	0.0066	0.0242	0.0014	0.0029	0.0019	0	0
		F x E	0.0394	0.0097	0.0357	0.0021	0.0043	0.0028	0	0

[1]Allzyme BG (1.0 mg/kg)

Excreta pH increased ($p < 0.05$) from the d 5 (trial diet application) to stability from d 10-19 in the starter phase on the wheat-based feed (Table 60). In the grower phase on the wheat diet (Table 61), excreta pH was greater ($p < 0.05$) when the birds were fed the whole-grain feeds and enzyme inclusion produced a higher ($p < 0.05$) pH.

The barley diet produced an increase ($p < 0.05$) in excreta pH from the start of the trial (Table 62) and considerable variation in the grower phase (Table 63). The whole-grain feed produced a lower ($p < 0.05$) excreta pH in the starter phase but enzyme inclusion had no effect ($p > 0.05$). Conversely, whole-grain inclusion or enzyme application produced a higher ($p < 0.05$) excreta pH as with the wheat diet.

Caecal evacuation pH on the barley diet (Table 64), whilst displaying variation over the course of the grower phase, was much moderated in comparison with the excreta pH. Whole grain inclusion produced a higher ($p < 0.05$) caecal content pH than the ground diet. Enzyme inclusion had no discernable effect ($p > 0.05$).

Short chain fatty acid concentration (mMol/L) (Table 65) and proportion of total SCFA (Table 66) were not significantly altered ($p > 0.05$) by grain processing or enzyme addition although eight replicates per treatment would have limited the success of finding statistically significant results.

10.4 Discussion

The principal aim of these experiments was to develop methodology of pH measurements of both excreta over time and of digesta as close to the time of euthanasia as possible. As well, the gas chromatography method for SCFA measurements required development and validation. The possibility of expanding the work to use a different type of bird and to test effects of cereal processing methods were sufficiently useful for the work to be done.

The differences in gut organ digesta contents, although different with each cereal, provided an indication that the use of a coarse fraction of cereal included in the pellets may cause food to be held in the gizzard for a longer period. This may simply be due to a large fibre fraction causing the gizzard musculature to work harder and thus form a larger, stronger gizzard which holds more feed, results in prolonged soaking of the digesta and, hence, initiation of the cereal grain endogenous enzymes and, finally, result in greater feed grinding in a stronger organ. This suggestion is supported by the greater digesta content in the gizzard and less in the duodenum of the barley-fed birds given the diets with whole-grain inclusion. The wheat trial data do not support this suggestion but may, indirectly, provide some support if grain size is considered. For consistency, each cereal type was processed in the same way through the same equipment. Grains of differing sizes are fragmented in different ways when processed through the same hammermill screen and pellet die size. Overall, the response to larger fibre particles should be consistent as the broilers were exposed to continuous light and, therefore, continuous access to feed.

The lower excreta pH on the ground barley diet with enzyme addition may support the above suggestion as finer material passes through the digestive tract more quickly than coarse material and thus more acid should be moved further down the tract as the co-ordinated back-flushing of digesta from the duodenum towards the gizzard may be reduced. As the pancreatic fluid is alkaline, if back flushing of digesta is reduced then the duodenal pH would be more acid. This may lead to a delay in amylase activation as most amylases, whether endogenous bird (Gapusan *et al*., 1990), endogenous cereal or many micro-organism amylases, function optimally at near-neutral to alkaline pH. Two conclusions may be drawn from these points; firstly, the lower pH was not due to fermentation or, secondly, that there was an increase in more intact grain fractions into the ileum and more fermentation lower down the gut.
Exogenous feed enzymes are in part promoted for their effect in reducing wet excreta problems. The higher excreta moisture produced by the supplemented wheat-fed birds was unexpected although the

moisture content was in the normal range of 75-80% (Larbier and Leclercq, 1992). Diarrhoea in birds may occur with the use of "problem" grains due to the viscous digesta holding water which is then lost with faecal material.

Although the digesta viscosity was minimal with the wheat and barley diets the feed enzyme significantly reduced the values obtained from the wheat diet. If feed turnover is enhanced with enzyme application perhaps some slight increase in water loss may not be unexpected and is of little concern when excreta moisture is within acceptable limits.

Excreta pH displayed no hint of any sudden reduction after the diet changes. It must be concluded that no excessive fermentation occurred with exposure of the gut to a novel feed. There were no signs of problems caused by the different cereals. Feeding well-aged grains produced in a good growing season resulted in little difficulty of the gut of relatively immature birds and their attendant microbial populations to adapt to a sudden feed change. With a poor type of grain, with high NSP levels for example, an increase in soluble material lower in the ileum or caeca or even the colon may lead to fermentation. Whole-grain inclusion producing a higher pH over the grower phase may be due to the feed being more effectively digested when being held for a longer period higher in the gut or may have an effect on micro-organism types in the lower gut. Alternatively, the ground grain fractions may be more readily fermented and with whole grain feeding more intact starch/other fractions may be lost in the excreta.

Although no significant differences in SCFA concentration were found the small number of replicates were of concern, although the same as used by Choct *et al*., (1996), as some patterns of numerical differences highlighted some distinct patterns of fermentative action. There was a suggestion that caecal SCFA production was pronounced with enzyme addition and a curiosity is apparent with the predominance of acetic acid production and minimal propionic and butyric acids. This does not follow the general pattern found in other species or in poultry trials where the C3 and C4 acids are produced in greater molar proportion (Corrier *et al*., 1990; Carre *et al*., 1995; Williams *et al*., 1997).

One result in the current broiler trials was of particular note. The inclusion of a proportion of the cereal fraction of the formulation as a whole-grain prior to pelleting resulted in a consistent reduction in proventricular dilatation (data presented in publications). There was also a significant reduction in deaths due to ascites, manifest as either right ventricular failure or "water-belly" (data presented in publications). When ascites mortality data for the full series of four broiler trials was subjected to Hazards Analysis by Cox's method (Cox, 1972) the relative risk of death was significantly ($p < 0.05$) reduced to 0.547 i.e. almost half in birds given a diet with whole-grain included in the pellets than when given a fully ground diet. When three different cereals i.e. triticale, wheat and barley, were considered at 20% inclusion as whole grain in the ground sorghum basal diet alone (the wheat inclusion on a wheat-basal diet removed from the analysis) the figure was reduced to 0.5. i.e. the relative risk of death due to ascites was exactly halved ($p < 0.05$). The manipulation of the cereal form had a positive therapeutic effect upon a specific condition and so the diet can be considered to have acted as a functional food.

11. General Discussion

Experimental methodology and feeds

The current project developed from work completed for an EIRDC post-graduate project undertaken by the researcher. At the completion of that project, several trials involving the use of both soluble and insoluble grits in broilers were done (Jones and Taylor, 1999). These trials were largely stimulated by observation of other work during voluntary labour contributed by the researcher to other RIRDC projects and contract trials involving investigation of grain characteristics and or feed enzymes in broilers. The commonly used methodology employed in AME work involves the growing of broilers to 21 or 24 days on commercial, steam-pelleted diets. These diets are generally based upon a blend of several cereals. The birds are then given the test diets for an "adaptation period" of three to four days until measurements are taken for the subsequent four days. The measurements include feed intake and weight gain and apparent metaboliseable energy is determined from the gross energy of the feed and the excreta. The excreta having been collected every 24 h and immediately oven-dried to minimise energy and other losses due to a host of reasons including fermentation. Although the methodology is subjected to criticism (Choct and Hughes, 2000) there are many factors associated with the feed preparation that may compromise the method directly but which are, apparently, ignored.

These factors were, in part, the reason for the additional broiler trials undertaken for this project. The broiler trials also allowed for procedures and methods to be developed and validated for the current layer project.

Relevant factors associated with feed processing included questioning why, with birds grown from day old on a fine-ground, steam-pelleted diet based on a blend of different cereals, test diets in AME trials were based on a single cereal that was cold-pelleted as a whole grain. As well, the diet formulations were based on inclusion of the single cereal at a constant level i.e. no account being taken of the protein and other contents. It may be valid to discount the energy content of the cereal (the utilisation of which is be estimated) but another level of error is added with the diets not being *iso*-nitrogenous diets. This is the simplest possible criticism; it does not consider differences in amino acid composition or starch content. Oddly, however, the often minute differences in moisture contents of each grain sample are factored into the calculations.

In the current broiler trials, simple consideration of the physiological effects of altering grain processing was targeted. In part, the results suggest (Jones and Taylor, 2001 attached) that other trials with enzymes may be compromised in short-term trials with AME measurement incorporated, by causing an adjustment period in the bird with a temporary reduction in growth/feed conversion efficiency and which far exceeds the test period. In other words, the initial "adaptation period" provides for a negative response that may "weight" results for a positive effect of the test enzyme.

The subsequent layer trials were originally planned to determine differences of biochemical markers of acid accumulation in the digesta. Therefore, the first of the current layer trials was designed to find if substitution of the commercial grower feed with a single-cereal-based feed, utilising the main cereal of that commercial feed, would cause a reduction in these markers. The commercial diet was used as a control. As the commercial diet returned a consistent excreta pH and the wheat diet significantly reduced excreta pH and digesta pH in the ileum and colon, largely the basis of the proposed trials, the use of the commercial diet was discontinued in several subsequent trials. With limited resources available for a speculative project, it was determined that comparison of a wide range of cereal types may lead to differences in lower gut digesta and excreta characteristics being found. Rice was subsequently to be used as the control diet as it has a high starch content largely unencumbered with substantial amounts of many other fractions considered to be involved in poor feed performance. The non-starch polysaccharides being considered prime amongst these fractions.

Furthermore, as all the feeds were cold-pelleted and crumbled, resulting in at least partial "cooking" of the feed, the physical difference with the commercial diet would be somewhat reduced. The methodology was determined by accepted practice with regard to comparisons of single cereals in poultry diets. In the final two experiments of the current work, the commercial diet was the basis for the work i.e. inclusion of enzyme or alterations to feeding methods suggested that the commercial diet should be included. This was also influenced by the use of an alternative feed provided by another miller and importantly, consideration of the earlier results which clarified some queries about the approach to the work. In the first layer trial, other results such as plasma and digesta lactic acid concentrations, whilst not being significantly different, were sufficiently confusing when considered with later results to cause doubts as to the validity of removing the commercial diet from the protocols.

The original aim was to determine if accumulation of acid, as organic acids, could occur within a short period in the lower gut of the laying bird exposed to a new single-cereal-based diet. In hindsight, the commercial diet should have been included in each trial to monitor if external changes were affecting digestion of this diet. The conclusion has been that any trial methodology involving comparison of single cereals should include the commercial growing diets used as a fixed control. Although commercial diets are generally based on a blend of cereals, there is the possibility that a form of "pre-conditioning" to these cereals may then exacerbate subsequent differences across a range of cereals for those cereals not found in the commercial diet.

In the current layer trials 1 to 5, the commercial diets were a wheat-sorghum blend and the wheat was itself a blend. The company has these wheat blends routinely subjected to AME determination by commercial arrangement with a tertiary institution. The 2000 season wheat had AME determined to be approximately 12.7 MJ kg^{-1} and this was considered to be consistent throughout the season i.e. whether new season (late 1999/early 2000) or old season (to the end of 2000) grain and this value was used for commercial formulations. As well, the diet was little changed over the year 2000. For the new season grain used in Feb 2001 the value was 12.6 MJ kg^{-1}.

As an aside, it was of note that at least one commercial layer producer who also grows substantial quantities of grain (wheats of several cultivars, sorghum and barley) was, in detailed discussion, interested in the commercial blend containing 60 percent durum wheat. Comment was made that there was a "fear" of using durum wheats in layer diets. Furthermore, the performance of the broilers on the "pure" wheat diets in the additional trials was very good which further allayed these "fears".

There was considerable criticism of the methodology employed in these experiments. The criticism was largely directed at the feed formulations/diets presented and an apparent lack of "control" diets. The diets were formulated in several ways prior to a final decision being taken for the feeding protocols. Firstly, the researcher discussed requirements with two commercial poultry feed nutritionists. Feeds were then formulated independently by the researcher and the commercial nutritionists; layer and broiler trial diets separately. There were two requirements; highest possible inclusion of a single cereal in each feed with specifications for the particular bird type being met (inclusion of each cereal had to be "forced"). This resulted in inclusion of other raws at levels that would not be found in the field. However, it is contended that failure of any diet to meet the breeders' recommended specification for their birds would add an unacceptable additional confounding factor to the experiments. This is another criticism of the standard AME methodology. There are many instances in the literature where minor raws e.g. fats, are included at differing levels in diet comparisons. In starch digestion trials in broiler chickens Weurding *et al*. (2001) used diets with varying inclusion of both soybean oil and animal fat; a brown rice-based diet used soybean oil with no added animal fat which had been included in all the other starch-source diets. These researchers saw no need of comment of such differences in potentially affecting digestion of starch from different sources.

In the field, commercial diets are generally constituted of blends of several cereals; seldom are single cereal diets used. The use of a single cereal diet could then be considered to be a novel feed.

Defense of the current methods could simply be limited to consideration of "standard" experimental methodology where commercial diet constituents are generally not specified nor are they often included as controls. However, with the constraints imposed on the current trials, it must be acknowledged that interactions of the other diet constituents may have influenced some results.

The hindgut and acidosis in the layer

Hill (1983) included the caeca and colon under the general term hindgut but indicated that the lower ileum functionally was part of the hindgut through the major innervation provided by the intestinal nerve. Some researchers have referred to the distal ileum as being "hindgut" in work that attempted to differentiate ileal effects of bird age and feeding regimen with wheat and barley (Petersen *et al*., 1999).

The hindgut of the chicken, generally deemed to consist of the caeca and colon, is small in comparison with that of most other animals and its importance in the contribution to total nutrition, energy in particular, is deemed to be of little importance. Furthermore, functionally the colon acts very differently to that in other animals with an almost constant retro-peristalsis (other than during defaecation and caecal evacuation) which appears to redirect nitrogenous products from the kidneys back to the caeca where micro-organisms utilise the N for their metabolic processes and which process may contribute to the birds' metabolism.

Starch does not just occur in different granule sizes but may be structurally different and is encapsulated, to varying degrees, by different proteins in the different cereals. The methods of grain processing (milling through different screens), pelleting (temperatures, die sizes and/or steam conditioning and moisture addition) will affect the degree of starch gelatinisation and subsequent "resistant" starch formation. Cooling, even subsequent to the relatively mild temperatures created with cold-pelleting, will alter starch structure through retrogradation. It is possible that in the current trials, the starch in the rice diets, with little fibre to interfere with starch exposure to the gut digestive processes, could be greatly affected by the processing to produce a greater degree of this type of resistance compared with the other cereals.

Similarly, native cereal starch is quite resistant to pancreatic α-amylases (Tervilä-Wilo *et al*., 1996) and these amylases themselves are of various types with respect to their activity in cleaving starch molecules. Taylor (1998) found differences in α-amylase activities in two layer strains fed the same diets. With the effect of gut micro-organism populations and their inherent amylases is the potential for different microbial populations to be introduced to the gut on the different cereals (which occurs with individual batches of the same grain). With the cold-pelleting process used in the current trials the effect of microbial population alteration may be a consideration although the limited period of data collection may preclude any great influence of population change and/or proliferation. The point must be considered as Jayne-Williams and Fuller (1971) were satisfied to quote a series of earlier workers in indicating that lower gut microbial populations were merely a passage flora of those established or predominant in the higher gut organs.

Efficient gut function and the attendant microbial population is affected by the various feed fractions. The use of exogenous enzymes, generally to cleave fibre fractions such as NSP's and so to reduce digesta viscosity to expose starch to enzymatic or acid hydrolysis higher in the gut may then affect the function of the hindgut. The caecum in most animals is the site of fermentation of fibre fractions but in the chicken the caecum is believed to be capable of receiving only very fine fractions (Hill, 1983) and the breakdown of NSP's higher in the gut may remove a valuable source of fermentable substrate for the resident microbial populations which are utilising N from the retrograde provision of uric acid and other nitrogenous wastes from the urodeum. The, albeit small, concentration of *iso*-forms of butyric and valeric acids found in some of the current experiments indicates that protein fermentation is occurring and is indicative of the levels of amines, phenols and indoles, shown to have potentially deleterious effects on gut health, that may be formed (Macfarlane and Macfarlane,

1995). Although microbial metabolism of proteins, of endogenous origin and/or derived of feed and/or micro-organism breakdown, may occur normally, the loss of any fermentable carbohydrate prior to digesta entering the caeca and colon may cause excessive protein fermentation by and/or favour establishment and/or proliferation of microbial types which have metabolic wastes with adverse effects thus far little considered in poultry.

Whilst positive effects on colonocytes of butyrate production (the preferred energy source for such cells) have been detailed, the effects are reversible (Kruh, 1982). Whilst effects are found at levels as low as 5-7 mM (Kruh, 1982), at these levels the concentration of other VFA's, largely acetic, found in the current trials, are at levels which, as well as contributing to a substantial total acid load in the gut, can effect negative changes in the mucosa. Furthermore, Kruh (1982) indicated that the more positive effects of sodium butyrate, centred on reversion of transformational characteristics and arrest of cell proliferation, on cells in culture, occurred in a limited number of cell lines.

Many gut anaerobes form lactate (either D- or L-) but it is not a major intermediate in fermentation and little is usually found in the gut. When large quantities of substrate flow through the gut and flux through glycolysis is great, lactate production is favoured and more fermentation lowers pH which inhibits the metabolism of microbial types which utilise lactate (Cummings, 1981).

Caldarini *et al*. (1996) described D-lactic acidosis in humans with short bowel syndrome. This was characterised by metabolic acidosis with elevated D-lactate in serum and urine. The condition was caused by carbohydrate malabsorption in the short intestine with subsequent fermentation by colonic bacteria to D-lactate which either accumulates in the gut and, if absorbed, is only slowly metabolised as D-lactic dehydrogenase is lacking. The condition was equated with lactic acidosis in ruminants although it was of note that with almost exclusively acetic acid being produced, a colonic pH of 4.8 and D-lactate at 59.6 mmol/l versus L-lactate at 12.7 mmol/l was produced in a 10 year old child. The plasma L-lactate was normal at 1.02 mmol/l but the D-lactate, which is not normally detectable, was 6.1 mmol/l. The level of plasma lactic acid in the layer is much higher than in most other animals (Bell and Culbert, 1968) and in healthy broilers levels of (the L-isomer at least) 7.6 mmol/L (calculated from the value of 7.6 μmol/mL presented in the paper) (Balnave *et al*., 1977) would be indicative of a severe metabolic acidosis in most other animals. Whilst levels of D-lactic acid were negligible in the plasma of the birds in most of the current experiments, the concentration in the gut segments achieved levels consistent with those considered problematic in other animals. The levels in individual birds was such that adverse tissue effects could be considered possible. Oh *et al*., (1979) described the formation of the L-lactate form derived from pyruvic acid and its subsequent metabolism via the L-isomer-specific lactate dehydrogenase and indicated that no animal possesses both D- and L- forms of lactate dehydrogenase. The presence of even minor concentrations of D-lactic acid are of concern metabolically and it is not known whether the bird possesses both L- and D-lactic dehydrogenases. The ability for secretion of sufficient bicarbonate in the colon to buffer acid could be overcome with excess fermentation and luminal pH could fall thus exacerbating acid accumulation by reducing lactate utilisation by microbial populations affected by acid conditions (Caldarini *et al*., 1996).

The accumulation of a large total organic acid load may lead to subtle and longer term negative effects on the mucosa; either enterocytes higher in the ileum or colonocytes in the hindgut. The current acceptance of the putatively positive effects of butyrate production for energy supply to the colonocytes is inconsistent with other results whereby damage to the gut accrues through excessive organic acid load. Total organic acid load (the VFA's plus lactic acid) may be great enough to cause damage at the cellular level that is not necessarily visible upon post-mortem inspection of gross anatomy. Rowe (1999) made speculative claims that the acid load at which potentially negative effects accrue for gut function and/or cellular integrity is not known and requires investigation. Another claim of Rowe (1999), again in a speculative context, was that chickens, being the only animal selected for so many generations on high cereal diets, have been indirectly selected for efficient digestion of starch. This could lead to the inference that, therefore, some sort of speedy

evolutionary diminution of the potentially damaging effects of high carbohydrate load in the birds' gut had occurred. The claim is interesting in that chickens are regarded as naturally granivorous (Sykes, 1983) so that inadvertent development of, or selection for, specifically, highly efficient starch utilisation has been part of real evolution of the fowl. This is especially reinforced if one considers the dietary habits of the progenitors of the modern commercial fowl, such as red jungle fowl, which may eat little more than grain at times in the yearly cycle (Collias and Collias, 1967). Given the effects of exogenous enzyme application and, at times, consistent positive responses to these commercial products, the gut of the commercial bird has not necessarily been positively adapted to improved starch utilisation through recent commercial genetic selection. One counter to the selection for starch utilisation argument is that it has apparently not been associated with efficient digestion of more complex carbohydrates. Further, the effect of sudden changes of cereal type with or without attendant changes in micro-organism loads is largely unknown. The above points are further confused by the suggestion of Pettersson and Åman (1989) that bacteria and endogenous grain enzymes are primarily responsible for fibre degradation in the crop and gizzard of the bird. The work with broilers in the current project indicates that positive responses can occur with some whole grain inclusion but that a relatively long period of gut adaptation, possibly as long as 25 d is required. This in itself opens to question the methodology employed in much poultry work where birds are allowed a mere three or four days to "adapt" to test diets, many of which are cold-pelleted, whole-grain based feeds; a point strongly made by Preston *et al*. (2000) and which would encompass the "classical" AME methodology.

There was evidence that, at times, accumulation of lactic acid occurred in the hindgut of layer-type birds when they were exposed to new single-cereal-based feeds. This accumulation was influenced by the quantity of feed consumed at different phases of the production cycle. Any increased water intake, due to heat stress or disease, may result in a similar increase in fermentation metabolities as digesta transit time may be reduced. Some consistent responses, deemed to be of concern for commercial poultry nutrition, to the change in diet were consistent throughout the course of this project and lead to application to study these effects in subsequent work.

Practical considerations

The results of this experimental project suggest several initial practical additions to feed testing methodology. Adaptation periods of three or, at times, four days, which are normal practice in AME trials, are substantially inadequate. Perhaps all test feeds should be fed from day one until the excreta collections are made. Additionally or alternatively, given criticism of the methodology used in the current experiments, if a commercial diet is used to grow birds to test age feeding of these diets should continue as a control. Although many trials are designed as factorial experiments, statistical analysis of factorial trials with an added control can be accommodated (Genstat has a facility for such a design). Secondly, simple measurements of pH change in fresh excreta may allow for monitoring of complex alterations in digestive processes during "adaptation" and collection periods. Such changes may be associated with altered microbial activity. pH measurements could be incorporated as part of standard methodology in all trials involved with comparisons of poultry feeds.

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Plain English Compendium Summary

Project Title:	
RIRDC Project No.:	UNC-12A
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Objectives	To provide evidence of hindgut acidosis in laying birds and to determine strategies to reduce or eliminate this condition
Background	Acidosis due to fermentation of carbohydrate in the hindgut of many species causes many acute and chronic conditions which lead to disease in the animal, with subsequent reduction of productivity, and environmental effects from water, gas and nutrient loss. Little research has been devoted to assessing whether any such condition could be caused in poultry. Given the contribution of cereal to standard diets, the substrates that provide for a potential fermentation problem could lead to similar difficulties in birds.
Research	Layer birds at different phases of the rearing and laying cycle were fed commercially practical diets but with inclusion of single cereals at maximal levels. Grain processing and alternative feeding methods were investigated as was feed enzyme inclusion. Responses of the digestive process were monitored to determine fermentation conditions and changes in digesta and blood pH. Additional broiler trials were undertaken to support this work and to provide any evidence of fermentation effects caused by alternative methods of grain processing.
Outcomes	Evidence was presented to show that fermentation can lead to accumulation of lactic acid in the digesta when birds are suddenly presented with different cereals. There was little evidence that, in the short term, a metabolic acidosis is incurred with such changes. A different wheat used in a second group of birds produced substantial increases in both SCFA and lactic acid accumulation in the ileal and caecal digesta of birds. Although the method of presenting the feed had little influence on acid conditions, application of an exogenous enzyme ameliorated the effects of lactic acid accumulation. In general, plasma pH consistently decreased over the 48 h trial periods which may suggest that the buffering capacity of the blood was being steadily reduced with an acid load. The broiler trials indicated that inclusion of some whole grain in pelleted diets may obviate the need for at least some feed enzyme inclusion. More importantly, the number of birds displaying proventricular dilatation was greatly reduced and the risk of death due to ascites was lower with whole grain inclusion in the feed.
Implications	The effects of sudden cereal change should be considered to have the potential to cause chronic effects of an acidosis in poultry. These effects may be very different to those that are addressed by feed enzyme application and may require further consideration of the need for growth promoters including available antibiotics. The processing of feed ingredients can have direct production effects that may involve fermentative and acidotic responses that require consideration at the time of feed formulation.
Publications	Taylor, R.D. and Jones, G.P.D. (2000). Production and physiological responses of broilers to the inclusion of whole grain into pelleted diets. Proc. Nutrition Society of Aust. Vol. 24. pp. 82-85. Taylor, R.D. and Jones, G.P.D. (2001). The effect of whole wheat, ground wheat and dietary enzymes on performance and gastro-intestinal morphology of broilers. Proc. Aust. Poult. Sci. Sym. Vol. 13. pp. 187-190. Jones, G.P.D. and Taylor, R.D. (2001). The incorporation of whole grain into pelleted broiler chicken diets. I. Production and physiological responses. British Poultry Science. **42**: 477-783.