INTERRELATIONSHIP OF DIET FIBRE AND ENDOXYLANASE WITH BACTERIA IN THE CHICKEN GUT

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ABSTRACT

Four trials were conducted to assess the influence of non-starch polysaccharides (NSP) from dietary corn (C), wheat (W) and wheat supplemented with endoxylanase (E) on performance, the gastrointestinal tract and on the bacterial population and its fermentation characteristics in broiler chickens. Trial one determined the difference in GIT size, specific culturable intestinal bacteria numbers, and volatile fatty acid (VFA) production at 42 d between C, W and wheat diets supplemented with E either throughout the 42 d or for 0-28 d or 29-42 d. The second trial utilized the same wheat treatments as the first trial, but GIT and VFA measures were taken at 14, 28 and 42 d while bacterial enumeration was only done at 28 and 42 d. This experiment was analyzed as a two-way analysis of variance with age and treatment effects. A third trial evaluated the C, W and E diets for differences in the amounts of total aerobic and anaerobic bacteria cultured at 28 and 42 d. The fourth trial was a two-way analysis of variance evaluating the effect of age and diet (C, W and E) on performance, GIT size, VFA production and residual NSP in the terminal ileum at 7, 14, 21, 28, 35 and 42 d. Performance was measured in all four trials and digesta viscosity was measured in all but the third trial.

Results from the first trial showed that E supplementation of wheat diets improved performance. Viscosity was lowest for C diets. Measures of GIT size were all smaller on C versus wheat-based diets. Ileal anaerobes tended to be higher with E than without while caecal anaerobes were higher on unsupplemented wheat diets. VFA production was higher for wheat versus corn fed birds in the ileum. C diets and wheat diets where E was removed at 28 d yielded the highest caecal propionic acid levels.

In the second trial, performance was also improved with E supplementation. Viscosity was lower for E supplemented wheat-fed birds than unsupplemented birds, except at 42 d. Full ileal weights were higher for W diets versus all others while caecal weights were lower on this diet. Bacterial data indicated higher levels of ileal anaerobes and some caecal anaerobes on W diets at 28 and 42 d. VFA content of the digesta, at 28 d was higher in the ileum in diets without E and the same tendency was noted for caecal

VFA. At 42 d ileal VFA production was higher with E and caecal VFA production was higher without E. The results from the first two trials demonstrate that while certain anaerobic bacteria do increase in the ileum of W diets, others appear to respond to the substrates created by E supplementation in both the ileum and the caecum. Age related adaptation also appears to affect the response of the bacteria to E supplementation.

In the third experiment C and E birds performed equally well with W birds having the highest gain to feed ratios after 14 d and overall. E diets resulted in the highest numbers of caecal anaerobes with C birds having the lowest number. At 42 d, birds had higher numbers of caecal anaerobes than at 28 d. At 28 d, caecal aerobes were highest on E diets ($P<0.10$) while at 42 d, caecal anaerobes were lowest on the C diet and similar for the two wheat diets. Therefore, despite performance similarities between C and E birds, there were definite differences in the bacteria present in the hindgut on each diet. This is likely due to the difference in residual dietary substrate in the hindgut of the birds fed different diets and its ability to enter the caeca. The substrates present in the ileum of E birds may be of benefit to both the bird and to the different cross-section of caecal bacteria present. Less NSP substrate is likely to be available in the hindgut of C birds.

Results from the fourth trial showed that performance was equivalent across treatments. Jejunal viscosity was highest ($P<0.05$) for the W diet at all ages except 7 d. Ileal viscosity was highest in this diet at all ages. Jejunal and ileal weights as a proportion of body weight were generally largest for W followed by E, and smallest for C. Caecal lengths followed the same pattern but caecal weights were highest for E. Ileal VFAs were not affected by treatment. Caecal acetic acid was highest for birds fed W and E diets, whereas caecal pH, propionic, isobutyric, isovaleric and valeric acids were highest for C birds. There was a significant interaction between diet and age for propionic acid. E diets had the highest ($P<0.001$) amounts arabinose and xylose from soluble and low molecular weight NSP present in ileal contents. As the birds aged, proportionally more arabinose and xylose was solubilized from the W diet. The E diet yielded higher, but relatively steady levels of soluble arabinose and xylose whereas the C diet yielded the lowest levels and no change was seen with age. This suggests a

bacterial adaptation to wheat NSP with age of the bird and the presence of NSP resistant to ileal bacterial hydrolysis in C diets.

It is concluded that the higher NSP content of the wheat diets is likely associated with the increased GIT size of the wheat-fed birds. While the NSP in these diets are broken down by the caecal bacteria to acetate and butyrate, the corn diet resulted in the production of propionate and isovalerate. The latter finding suggests that undigested starch and protein from corn enter the caeca and are being fermented by bacteria in this location. All of the differences in bacterial composition, fermentation and substrates provide evidence for changes in dietary NSP content and structure having a significant impact on changing the bacterial profile of the GIT of the broiler chicken.

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This thesis is dedicated to the purveyor of the concept that God only gives us as much of a challenge as we are able to handle. I now know this to be true.

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LIST OF ABBREVIATIONS

AME	apparent metabolizable energy
AMEn	nitrogen-corrected apparent metabolizable energy
Ara	arabinose
AX	arabinoxylan
CFU	colony forming unit
CP	crude protein
DE	digestible energy
Gal	galactose
GC	gas chromatograph
GI	gastrointestinal
GIT	gastrointestinal tract
Glc	glucose
Man	mannose
ME	metabolizable energy
NDC	non-digestible carbohydrate
NSP	non starch polysaccharide
OS	oligosaccharide
SI	small intestine
UA	uronic acid
VFA	volatile fatty acid
Xyl	xylose

1.0 INTRODUCTION

Cereal grains comprise the bulk of western Canadian poultry rations, as they do in much of Europe and Australia. Since these grains are known to contain variable levels of anti-nutritive dietary fibre or nonstarch polysaccharides (NSP), accurate ration formulation is often difficult. While much is known about the chemical composition of intact dietary NSP and their physiological effects in poultry diets, less detail is available on the physiological and microbiological effects of enzymatically degraded NSP or the interactions between gut microflora and dietary NSP size, composition and level.

Long chain length carbohydrates (NSP) can decrease nutrient availability through a number of mechanisms, including increased digesta viscosity and microbial proliferation in the hindgut. At the opposite end of the spectrum, some shorter chain length carbohydrates (specific mono and disaccharides) are poorly absorbed in the lower small intestine, cause high osmotic potential in the digesta and, again, result in increased microbial fermentation. It is known that certain genera of native gut microflora negatively influence nutrient availability, while others appear to be beneficial. The same may be said about different sized carbohydrate fractions (polysaccharides vs oligosaccharides vs di- and monosaccharides).

There is, however, ample evidence of performance improvements with enzymatic degradation of NSP. The use of endoxylanases in wheat and ß-glucanases in barley diets is common practice in feeding monogastric animals. The enzymes decrease

digesta viscosity, improve nutrient utilization, reduce variability in the ME of cereal grains and improve animal performance. Enzymatic hydrolysis of NSP results in the formation of smaller polysaccharides through depolymerization of the larger NSP. It may also result in the release of some oligosaccharides and monosaccharides.

Some research has demonstrated that enzyme supplementation of high NSP diets reduces the proliferation of the ileal microflora in chickens. Improved nutrient utilization may be related to the reduction of digesta viscosity and therefore improved nutrient flow and enzyme-substrate contact or it may also be due to the change in the microflora. Enzyme supplementation may also increase the proliferation of some caecal microflora, which may or may not be beneficial to the host animal. The interactions between the various carbohydrate fractions of different sized whole and degraded NSP and the microbial population have not yet been clearly elucidated.

The objectives of this research were to identify the relationship between dietary NSP and endoxylanase supplementation on numbers of specific groups of gut bacteria and on bacterial fermentation in the distal small intestine and caeca of poultry; to determine whether adaptation of the microflora to dietary NSP and endoxylanase supplementation occurs with time; and, to identify differences in the size and composition of NSP fractions which remain in the terminal ileum as affected by dietary NSP, endoxylanase supplementation and age, and to evaluate how this may impact gut microflora status.

2.0 LITERATURE REVIEW

2.1 Non-starch Polysaccharides (NSP) in Feed Ingredients

2.1.1 Components of Dietary Fibre

Non-digestible carbohydrates (NDC) are a combination of indigestible non-starch polysaccharides (cellulose and hemicelluloses) and other carbohydrates such as pectic substances and glycoproteins that make up plant cell walls. These products are usually considered the major constituents of dietary fibre although some schools of thought also include waxes, cutin, lignin and even resistant starch (Trowell *et al.*, 1976; Baker *et al.*, 1979; Theander and Åman, 1979; Brown, 1996), while still others include proteins, phenolic esters and gums from food additives (Selvendran, 1984).

According to Englyst (1989), the principal components of dietary fibre are the non-starch polysaccharides (NSP) of plants. In terms of animal nutrition and the practical application of the dietary fibre concept in feedstuff utilization, the definition of Theander and Åman (1979) is the most appropriate. Theander and Åman (1979) define dietary fibre as "A group of polysaccharides and other polymers in plant material in the diet which are neither digested by normal secretions nor absorbed in the upper gastrointestinal tract." This applies particularly well to animal nutrition because components of human foods such as additives are not normally a large part of animal feeds.

In keeping with the above definition, the carbohydrate constituents of dietary fibre are the cell wall NDC, which include cellulose, hemicellulose and pectic substances, as well as other non-structural plant substances (Baker *et al.*, 1979). Of the NSP, cellulose is predominant. Cellulose is a linear polymer of glucose with ß-1,4 linkages. Hemicelluloses, on the other hand, are primarily made up of ß-1,4 linked xylose residues, sometimes with L-arabinose or D-glucuronic acid residues attached to the main chain. Hemicelluloses may also include units of mannose, galactose, or fucose. Other mixed-link glucans present in some plants include such polysaccharides as ß-glucans with ß-1,4 linked glucoses interspersed with ß-1,3 linkages (Theander and Åman, 1979; Theander *et al.*, 1993). Pectic substances are a complex group of polysaccharides in which D-galacturonic acid is a principal constituent with residues of rhamnose, xylose, galactose, and fucose along with some uronic acids present as methyl esters.

Lignin, is also present in cell walls and resistant to digestion in both the small and large intestine. However, this amorphous, high molecular weight aromatic polymer is composed of phenyl propane (Selvendran, 1984) and is not a carbohydrate.

2.1.2 Chemical Composition of Non-starch Polysaccharides (NSP)

A closer examination of the chemical structure of NSP shows that they are composed of 11 or more of either one, or a combination of ten different monosaccharide units connected to each other through various glycosidic linkages (Theander *et al.*, 1993). The number 11 is merely used to differentiate between oligosaccharides (up to

10 units) and polysaccharides. The ten different monosaccharides can be grouped by number of carbons as well as by the presence of a hydroxyl, methyl, or carboxyl group on C-5 of the ring structure. The five carbon sugars (pentoses) are xylose and arabinose and the six carbon sugars (hexoses) are mannose, glucose, and galactose. The uronic acids are six or seven carbon structures with a carboxyl group on C-5 of the ring structure; uronic acids include glucuronic, galacturonic and 4-0-methyl glucuronic (7 C) acids. The deoxyhexoses are rhamnose and fucose and have a methyl group on C-5.

All of the sugars, except arabinose, occur predominantly in their pyranosidic ring or "chair" form with xylose, mannose, glucose and galactose occurring as ß-linked pyranosidic rings and rhamnose, fucose and all three uronic acids occurring as α-linked pyranosidic rings. Arabinose alone occurs as an α-linked furanosidic ring (Theander *et al.*, 1993).

The predominant NSP in cereal grains are arabinoxylans and ß-glucans which form an amorphous matrix around small but significant amounts of cellulose micro fibrils closely associated with glucomannans (Selvendran, 1984). The relative proportions of the major cereal grain NSP vary with species of plant where wheat, rye and triticale have predominantly arabinoxylans and barley and oats have mostly ß-glucans. In barley, ß-(1,3), (1,4)-glucans make up 30-60 g/kg DM (Fincher and Stone, 1986). In rye, arabinoxylans are present at around 100 g/kg (Antoniou *et al.*, 1981) and in wheat, they are present at anywhere from 50-80 g/kg (Annison, 1990). In wheat the arabinoxylans are largely located in the cell walls of the aleurone layer (Posner, 2000). In corn, arabinoxylans are present at anywhere from 43-66 g/kg but are mostly located in

the bran, not connected with the starchy endosperm and are largely insoluble (Choct and Annison, 1990; Shelton and Lee, 2000).

The actual monosaccharide breakdown of cell wall NSP from a number of different cereal grains were summarized by Chesson (1995) (Table 2.1).

Table 2.1 Monosaccharide composition of the cell-wall polysaccharides (NSP) from various grains (adapted from Chesson (1995) with corn data from Shelton and Lee (2000))

		Monosaccharides (% total NSP)					
Source	% DM	Ara	Xyl	Man	Gal	Glc	UA
Barley	15.0	14.7	25.1	2.7	1.9	51.8	3.3
Oats	27.9	7.1	33.7	1.6	2.4	50.5	4.2
Rice	2.0	21.3	34.2	---	5.4	42.1	6.9
Rye	13.7	22.1	37.0	4.5	4.2	28.4	3.1
Wheat	10.9	20.9	33.8	4.1	4.3	33.0	3.9
Corn	n/a	23.1	37.8	n/a	7.7	n/a	2.1
Peas (Dried)	21.5	18.6	8.5	0.9	3.7	58.1	9.3

n/a – not available

Pectic substances are a complex group of colloidal polysaccharides that may be partially extracted with water but which sometimes require the use of chelating agents in aqueous solution for complete extraction. This is due to the presence of calcium and magnesium ions (Theander *et al.*, 1989). As mentioned earlier, pectins are mostly methyl esters of D-galacturonic acid, which are usually substituted with rhamnose residues forming rhamnogalacturonans. Side chains of arabinose and galactose are often present. Cereal grains have relatively low amounts of pectic substances compared to most dicotyledonous plants (Theander *et al.*, 1989; Chesson, 1995) such as oilseeds. In canola meal, for example, rhamnogalacturonans comprise the largest proportion of NSP (Slominski and Campbell, 1990).

Proteins and phenolic compounds can be associated with polysaccharides in cereal grain endosperm and aleurone layers. The way in which NSP are associated with non-carbohydrate fractions in plant cell walls becomes important when the plant product is consumed. These associations can influence the solubility of the NSP and other factors that impact on their physicochemical properties in the bird's gastrointestinal tract (Smits and Annison, 1996) as well as on the availability of the nutrients in the plant product.

Oilseed meals used as protein sources in poultry diets often have substantial levels of free sugars and their α-galactosides which cannot be digested in the avian small intestine due to a lack of endogenous α-(1,6) galactosidase (Gitzelman and Aurichio, 1965). These oligosaccharides, called raffinose oligosaccharides or α-galactosides,

typically have galactose residues attached through an α-(1,6) linkage to the glucose moiety of sucrose and, depending on their degree of polymerization, can be present as raffinose, stachyose, verbascose, or ajugose (Veldman *et al.*, 1993). Of the raffinose oligosaccharides, stachyose is predominant in soybean meal and canola meal while verbascose is predominant in field peas (Bach-Knudsen and Li, 1991).

2.1.3 Physiological Implications of Non-starch Polysaccharides in Feed Ingredients

2.1.3.1 Physical Implications of NSP

Non-starch polysaccharides can to cause physical changes in the GIT environment. The branched nature of the water-soluble arabinoxylans gives them gel-forming properties, allowing them to absorb water and form viscous solutions in the digesta of poultry (Annison, 1993; Chesson, 1995). This increased digesta viscosity is considered one of the anti-nutritional factors associated with NSP. The increased viscosity can slow digesta passage rate (Salih *et al.*, 1991; van der Klis and van Voorst, 1993; Almirall and Esteve-Garcia, 1994), decrease access to the feed by the digestive enzymes of the bird (Johnson and Gee, 1981; Johnson *et al.*, 1984; Fengler and Marquardt, 1988a) and cause increased endogenous secretions in the GIT (Choct and Annison, 1992a; Angkanaporn *et al.*, 1994). In a review by Ellis *et al.* (1996) the author cites studies in dogs and pigs that demonstrate that dietary soluble NSP increases gastric viscosity thereby affecting sieving and mixing, resulting in larger sized food particles entering the small intestine. These authors summarize that soluble NSP increases SI

viscosity, thereby inhibiting the mixing of nutrients and enzymes by reducing the effects of intestinal contractions, increasing the volume of intestinal secretions, and possibly changing the pattern of digesta flow from turbulent to laminar (stream-line) flow. Other physical effects of dietary fibre, and specifically, of NSP, include an increase in GIT length and weight (Johnson *et al.*, 1984; Johnson and Gee, 1986; Moss, 1989; Savory, 1992a; van der Klis and van Voorst, 1993; Jørgensen *et al.*, 1996; Smits *et al.*, 1997). Increased thickness of the "unstirred" water layer was observed when everted sacs of rat jejunum were incubated with two sources of viscous NSP (guar gum and carboxymethylcellulose (CMC)) (Johnson and Gee, 1981). An increase in the proliferation rate of enterocytes of rats fed the same two sources of NSP was shown by Johnson and Gee (1986). Differences in pH and osmolality are also seen with increasing rates of NSP inclusion in the diet. Van der Klis *et al.* (1993) noted a decrease in pH in the ileum and an increase in ileal osmolality with increasing addition of carboxymethylcellulose in the diets of broiler chickens. Research has also demonstrated that both guar-gum and carboxymethylcellulose decrease levels of some mucosal enzymes while response of some other enzymes is not the same for the two sources of dietary fibre (Johnson *et al.*, 1984; Johnson and Gee, 1986). This led the researchers to conclude that the physical changes in the GIT that result from the feeding of dietary fibre relate to more than just the changes in the viscosity of the digesta.

2.1.3.2 Nutritional Implications of NSP

The viscosity generating properties of soluble NSP have been shown to decrease

the digestibility of protein, starch and fat (Smits and Annison, 1996) and to affect the energy value of cereal grains. Antoniou *et al.* (1981) worked to characterize the component of rye that was causing antinutritional effects and found it to be a water-soluble, pentosan-rich fraction of the grain. Fengler and Marquardt (1988b) further purified and characterized this component and used it in feeding trials with chicks. In these studies, fat retention by the chicks was shown to be adversely affected by the isolated fraction. Extractions were also made of purified wheat arabinoxylans, first on a laboratory scale (Choct and Annison, 1990) and then on a larger scale (Annison *et al.*, 1992), were conducted to obtain material with which to evaluate different anti-nutritive aspects of wheat pentosans in broiler chicken diets. Choct and Annison (1990) isolated >1000 g of arabinoxylan-rich material which had the same anti-nutritive properties as wheat. The second larger scale extraction (Annison *et al.*, 1992) was conducted to ensure that the material isolated in large quantities was indeed similar in structure and properties to wheat arabinoxylans in complete feeds and foods. This was confirmed using structural analysis and nuclear-magnetic resonance techniques. The isolated arabinoxylans were then used in further studies to determine the negative impact of wheat arabinoxylans on AMEn, nutrient digestibility, digesta viscosity and broiler performance (Choct and Annison, 1992a; 1992b).

Many years of research have been spent determining that it is a combination of the size, structure and solubility of the NSP in cereals that causes their antinutritional activity. Initially it was thought that the low AMEn values for some Australian wheats were attributable to poor starch digestibility and showed an improvement with increasing

age of birds (Mollah *et al.*, 1983; Rogel *et al.*, 1987). Further work demonstrated significant and highly negative correlations between AMEn and water soluble NSP (Annison, 1991) which, in wheat and rye, consists mostly of arabinoxylans. While some studies (Pettersson and Åman, 1989; Choct and Annison, 1992b) showed that the significant negative effect of cereal grain pentosans and ß-glucans on broiler performance was a result of increased digesta viscosity, Choct and Annison (1992b) confirmed that this physicochemical property of NSP, and arabinoxylans in particular, is dependent upon the degree of polymerization of the polysaccharide. Since degree of polymerization is related to molecular weight, Bedford and Classen (1992) conducted a study evaluating enzyme dose response on molecular weight distribution of NSP fractions from rye and wheat in the broiler GIT. They concluded that the concentration of a high (>500 kDa) molecular weight fraction alone was not responsible for its viscosity generating properties, but that the sugar composition of this fraction might also be partially responsible.

A number of years prior to this, Carré *et al.* (1984) went so far as to develop a prediction equation for AMEn based on the level of water soluble cell wall material present in a feedstuff and confirmed the accuracy of this equation through the evaluation of NSP digestibilities with cockerels, ducks and rats (Carré *et al.*, 1990). These researchers noted very little species differences in AMEn between ducks and cockerels but large differences between the two avian species and rats. The results were said to suggest that the main factor accounting for the variation in AMEn (or DE, for rats) between species was the ability to digest NSP. More recent work by Austin *et al.*

(1999) demonstrated that AME was even more closely related to the actual component of arabinoxylan resistant to hydrolysis by xylanases, and to the degree of branching of the arabinoxylan, than to the soluble NSP content of the wheats tested.

Digestibilities of fat, starch and protein in wheat were reduced when the wheats had high levels of arabinoxylans (Choct and Annison, 1992a,b; Choct *et al.*, 1992). Lipid digestion, in particular, is adversely affected by dietary pentosans (Campbell *et al.*, 1983; Fengler and Marquardt, 1988a) and other NSP (Smits *et al.*, 1997). This was found to be related to the microbial status of the bird and will be discussed in greater detail in Section 2.1.3.3.

Despite starch digestibility having been found to explain about 88% of the variability in Australian wheat AME (Rogel *et al.*, 1987), when isolated from the wheat, the starch was always 99.9% digestible, regardless of the AME of the wheat it was taken from (Rogel *et al.*, 1987). In further studies with higher AME wheats, starch digestibility was not correlated with the level or composition of wheat NSP (Annison, 1990). It was suggested that perhaps the NSP levels in this study were not at high enough levels to yield the anti-nutritive effects on starch digestibility. When isolated wheat pentosans were added to poultry diets at higher levels in the experiments of Choct and Annison (1992a,b) starch digestibility was again reduced. It was also reduced when other sources of NSP such as pea fibre or oat bran were used (Jørgensen *et al.*, 1996).

Protein digestibility is also reduced by NSP addition to diets, particularly with NSP that induce high digesta viscosity (Smits *et al.*, 1997). The same effect was seen in pigs fed high levels of ß-glucans (Bach-Knudsen *et al.*, 1993). In one study with poultry,

Angkanaporn *et al.* (1994) showed that the addition of low levels of wheat pentosans decreased amino acid digestibility largely by increasing endogenous protein secretions. In the same study, higher levels of pentosans decreased amino acid digestibility by impeding the breakdown of proteins and subsequent amino acid absorption, as well as increasing endogenous secretions indicating a dose-dependent response to dietary NSP.

Other dietary ingredients have NSP that can contribute negatively to nutrient digestibility. Many legume meals fed to poultry have high concentrations of raffinose oligosaccharides (α-galactosides). Soybean meal has 5.6% oligosaccharides (OS), field peas have 6.3% OS (Kuo *et al.*, 1988) and canola meal has 2.6% OS (Slominski *et al.*, 1994). As mentioned earlier, these OS cannot be broken down in the small intestine of poultry due to a lack of endogenous α-1,6-galactosidase (Gitzelmann and Auricchio, 1965). Their presence in the gastrointestinal tract has an effect on nutrient absorption for two reasons. First, α-galactosides are low molecular weight water soluble compounds which cause the digesta to have high osmotic pressure; and, secondly, they are readily fermented by intestinal microbes (Wiggins, 1984). In mammals, the high osmotic pressure of the digesta when α-galactosides are present causes an increase in fluid and electrolyte levels in the hindgut leading to faster digesta passage rates. Despite the expected and observed negative effect in at least one study with piglets (Veldman *et al.*, 1993) of α-galactosides on nutrient absorption, other studies have shown little direct negative impact in poultry diets (Brenes *et al.*, 1989; Slominski *et al.*, 1994; Irish *et al.*, 1995). The negative performance effects elicited by some ingredients high in α-galactosides have been suggested to be due to other factors such as high levels of pectic

substances (Carré and Leclercq, 1985; Brenes *et al.*, 1993) or of other ethanol soluble materials (Slominski *et al.*, 1994).

2.1.3.3 Microbial Implications of NSP

The increase in digesta viscosity caused by the presence of NSP in broiler diets has been shown to decrease digesta passage rate, thus increasing the likelihood of microbial overgrowth in the hindgut (Annison and Choct, 1991; Salih *et al.*, 1991; Choct *et al.*, 1996; Smits and Annison, 1996; Smits *et al.*, 1998). Microbial activity has been shown to be higher on high NSP diets as measured both by elevated production and concentration of volatile fatty acids (VFAs), the products of microbial fermentation of NSP, in the hindgut (Annison *et al.*, 1968; Choct *et al.*, 1995; Choct *et al.*, 1996) and by increased numbers of anaerobic bacteria in the small intestine (Wagner and Thomas, 1978; Langhout, 1998; Smits *et al.*, 1998; Langhout *et al.*, 1999).

The decrease in fat digestibility on high NSP diets is thought to be related to increased microbial activity. It has been shown that the negative effect of wheat pentosans on fat digestibility is lower in caecectomized versus intact chickens (Choct *et al.*, 1992). It has also been shown that added fibre and the resultant increase in digesta viscosity has no effect on fat digestibility in germ-free chicks (Smits and Annison, 1996). It is thought that increased bacterial activity in the hindgut may increase the deconjugation of bile acids thus impairing their return to the liver for recycling into bile. Coates *et al.* (1981) demonstrated both *in vitro* and *in vivo* deconjugation of bile acids by *Enterococcus faecium* isolated from chickens. Campbell *et al.* (1983) noted

decreased fat retention of rye-fed poultry raised either with a conventional GI microflora or germ-free and associated with *Enterococcus faecium* compared to wheat-fed or germ-free rye-fed birds. They also found that supplementation of the rye diet with sodium taurocholate improved fat retention, indicating that the bacteria were decreasing the availability of bile salts to the bird. Feighner and Dashkevicz (1988) took this premise one step further and were able to correlate intestinal levels of bile salt hydrolases with dietary NSP induced growth depression in poultry. These ideas were supported by Salih *et al.* (1991) who observed an improvement in fat digestibility as the numbers of *Enterococcus faecium* declined with age of the bird. More recently, Smits *et al.* (1998) demonstrated a reduction in bile acid concentration with inclusion of a non-fermentable dietary fibre (CMC) and this was associated with reduced lipid digestibility. They also noted a significant increase in excreted bile salts with CMC in the diet. In addition, Langhout (1998) demonstrated that feeding high-methylated citrus pectin (as a source of anhydrous NSP) resulted in increased deconjugation of bile acids in 22 day chicks. Deconjugation of bile acids was related to the increased proliferation of anaerobic microflora in the small intestine, which supports the previous studies.

In studies comparing conventionally reared chickens with germ-free chickens, Muramatsu et. al. (1991) demonstrated that conventionally reared birds derived higher metabolizable energy and had better fibre digestibility on high fibre, low energy diets than germ-free birds. This difference disappeared when birds were fed low fibre, energy adequate diets. Later, Muramatsu *et al.* (1994) conducted a study to look more closely at the relationship between the gut microflora and energy utilization by the chicken. The

researchers varied the level of ME intake from zero to above adequate and regressed heat production and energy deposition on ME intake. The microbial population of the hindgut was shown to reduce the efficiency of energy metabolism by decreasing energy deposition relative to ME intake as compared to birds without a GI flora. In the same study, it was also shown that birds with inadequate availability of dietary energy could derive benefit from the presence of gut microbes through a reduction of energy losses from heat production. The regression of heat production on ME intake was substantially different between germ-free and conventional birds indicating a buffering effect of the bacteria on the efficiency of energy use, depending upon energy availability. It was suggested that part of the loss of efficiency of energy utilization was due to the GI flora increasing energy costs by increasing the rate of energy requiring reactions such as protein synthesis (Muramatsu *et al.*, 1988). It has been shown that the GIT bacteria of birds fed diets with NSP ferment residual dietary substrates in the hindgut (Bedford, 1996a) and cause a reduction in the digestibility of dry matter, organic matter, starch, and fat, as well as a reduction in dietary ME (Langhout *et al.*, 2000). The bacteria were implicated since germ-free birds fed the same diets showed no decreases in digestibility and actually had an increase in dietary ME with added NSP. A further cause of the reduced efficiency of energy utilization by birds with higher numbers of GIT bacteria is attributed to their conversion of carbohydrate substrate into VFAs which are then, under conditions of adequate energy (Muramatsu *et al.*, 1994), largely excreted and therefore, not of use to the bird (Choct, 1999). Choct (1999) compared the excreted VFAs from chickens fed corn and barley and noted a significantly higher amount of energy from

VFAs lost from barley-fed birds. Bedford and Schulze (1998) review the many factors confounding the impact of NSP on the GIT microflora in detail.

2.2 Changes in Physiological Responses to NSP with Age

It has been determined in numerous studies that the anti-nutritive properties of cereal grains decrease as the bird ages (Classen *et al.*, 1985; Classen *et al.*, 1988; Salih *et al.*, 1991). At the same time, intestinal viscosity is decreased (Almirall and Esteve-Garcia, 1994; Petersen *et al.*, 1999) and nutrient digestibility is increased with age (Rogel *et al.*, 1987; Carré *et al.*, 1995). Speculation is that this is an effect of gastrointestinal tract development including increased endogenous enzyme secretions coupled with increased absorptive surface area and gut musculature and their effects on GIT residence time (Angkanaporn *et al.*, 1994; Smits *et al.*, 1997, Bedford and Schulze, 1998). However, this could also be a function of increased microbial proliferation in the GIT of NSP fed birds. Rather than a negative effect on efficiency of energy metabolism, perhaps the gut microflora make a positive contribution through NSP hydrolysis and a subsequent reduction in viscosity (Bedford and Morgan, 1996).

A closer look at age related differences in nutrient digestibility by Carré *et al.* (1995) showed that there was very little difference between broiler chicks and adult cockerels of a laying strain in digestibility values for NSP with low water solubility. They also showed that the digestibility of more water soluble substances such as pectin, α-galactosides and lactose were high in both species but were higher in the cockerels than in the broilers. It was concluded that the higher AMEn values for diets fed to adult

birds were a result of a higher digestibility of fermentable carbohydrates such as α-galactosides and of water soluble NSP.

Despite the knowledge that birds perform better on NSP-containing diets as they age, there is little published research specifically examining an age effect, therefore, there is little hard evidence linking dietary NSP with what is occurring in the gastrointestinal tract of the bird as it ages.

2.3 Normal GIT Microflora of Poultry

A review of the current literature reveals a limited number of recent works on the identification of the bacteria of the GIT of poultry. A comparison of two recent reviews supports the fact that little has been done in this area in the recent past (Mead, 1997; Mead, 2000). The actual organisms present have been partially characterized through years of research in the laboratory of Dr. Ella Barnes and her colleagues (Mead, 1997). Most estimates of numbers of different genera of bacteria present in the GIT of poultry are from 400-500 (Bedford and Apajalahti, 2001). Even so, it is estimated that less than 10% of the normal flora of the GI tract can be cultured using traditional methods (Apajalahti and Bedford, 2000).

2.3.1 Development with Age

The normal microflora of the poultry digestive tract develops rapidly in the foregut after hatch, within two weeks in the small intestine (Mead, 1997; Smith, 1965) and much more slowly in the caeca, where it takes about 6 weeks to establish an adult flora (Barnes *et al.*, 1972). The slowness of caecal flora development is a reflection of

the highly sanitized conditions in which commercial poultry are raised, without contact with parent birds or excreta (Mead, 1997).

2.3.2 Bacteria of the Crop

The actual types of bacteria present in the avian tract vary with the segment of the tract under study. The crop, which has a median pH of around 4.2-5.0, is colonized in a very thin, but fairly complete layer over its stratified, squamous epithelium predominantly by *Lactobacillus*. These stabilize at around 10^8 CFU/g and exert both bacteriostatic and bactericidal effects to control populations of *E. coli* (Fuller, 1977). Coliforms stabilize at around 10^4 CFU/g and Enterococci at 10^5-10^6 CFU/g. Some *Clostridium perfringens* can be found as well (Mead, 1997).

2.3.3 Bacteria of the Small Intestine

The bacteria of the SI are very limited in the duodenum due partially to high digesta passage rates. Numbers of aerobes and facultative anaerobes are similar in different segments of the SI with the predominant organisms being *Enterococcus, Staphylococcus, Lactobacillus,* and *E. coli* (Salanitro *et al.*, 1978; Mead, 1997). Obligate anaerobes present include *Eubacterium, Propionibacterium, Clostridium, Gemminger,* and *Fusobacterium* spp. (Mead, 1997). Strict anaerobes can make up anywhere from 9-39% of the total number of strains isolated in the SI although high variability between birds was noted (Salanitro *et al.*, 1978).

2.3.4 Bacteria of the Caeca

The caeca are the area with the most complex microflora within the avian GIT. Strict anaerobes comprise the bulk of the bacteria and are present at around 10^{11} CFU/g. The predominant bacteria are gram positive anaerobic cocci (28%) including various species of *Coprococcus, Peptostreptococcus,* and *Enterococcus*, with the remainder a mixture of gram negative, non-sporing rods (*Bacteroides* spp., 20%) and gram positive, non-sporing rods (*Eubacterium* spp., 16%) (Barnes *et al.*, 1972; 1979). *Gemminger formicilis* (5%), a budding bacterium, plus some budding cocci (6%), *Clostridium* spp. (5%), and *Bifidobacterium* spp. (9%) make up the bulk of the identifiable anaerobes (Barnes *et al.*, 1972; 1979). Facultative anaerobes are also present in the caeca throughout the life of the bird. Barnes *et al.* (1972) showed that coliforms and lactobacilli were present at 10^6 to 10^8 CFU/g while enterococci were found at 10^5 to 10^7 CFU/g after 4 weeks of age.

Most of the major anaerobic bacteria of the caeca are saccharolytic and nearly all ferment glucose with a surprising number also able to ferment lactose. Of interest is the fact that most caecal strains of budding bacteria and some other genera are able to grow on arabinoxylan (Croucher and Barnes, 1983; Mead, 1989). As mentioned earlier, many caecal bacteria are also able to degrade uric acid (Barnes and Impey, 1974; Mead and Adams, 1975), present in the caeca through retrograde peristalsis from the cloaca, although essentially none have been shown to have an absolute requirement for uric acid as a substrate (Barnes and Impey, 1974). Bacteria degrading uric acid cause large amounts of ammonia to be produced in the caeca which is quickly utilized as a nitrogen source by a high proportion of the caecal microflora (Karasawa *et al.*, 1988).

2.3.5 Negative Bacterial Impact on the Bird

In the normal intestinal tract of poultry the microflora exert both positive and negative effects. Early studies with germ-free birds indicated that the presence of gut microbes actually decreased the bird's ability to digest and absorb saturated fatty acids (Boyd and Edwards, 1967). The work of Cole *et al.* (1981) also demonstrated an increase in the apparent digestibility of lipids in germ-free versus conventionally reared birds, but did not confirm the improved absorption of saturated fatty acids. Other studies showed GI tract pH was lower in conventional than germ-free birds (Ford, 1974), attributing this to the VFAs produced by the bacteria. Growth depression and increased gut weight in the presence of bacterial challenge were also demonstrated (Coates *et al.*, 1981).

Early work on the growth promoting effect of antibiotics implied that the natural gut microflora must either include species or produce substances that cause growth depression in the host (Eyssen and De Somer, 1967). Attempts to isolate this negative factor concentrated on the species *Enterococcus faecalis* (Eyssen and De Somer, 1967) and *Enterococcus faecium* (Fuller *et al.*, 1979; Houghton *et al.*, 1981). It was shown in these studies that the growth depression effect seen with both *Enterococcus faecalis* and *Enterococcus faecium* was stronger when the organism was fed with a bacteria-free filtrate made from the excreta of conventionally reared chicks. This filtrate was also able to induce a growth depression effect when fed with bacteria other than *E. faecium* even when these bacteria could not induce a significant growth depression when fed on their own (Fuller *et al.*, 1979). In a separate study evaluating the growth promoting

effect of fish solubles in poultry diets it was postulated that the fish solubles somehow caused favourable manipulation of the bird's gut microflora (Harrison and Coates, 1972). This was suggested since the feeding of fish solubles overcame the substantial growth depression induced by feeding *E. faecalis* and a bacteria-free filtrate to germ-free chicks. Fish solubles had no effect on the growth of germ-free chicks that were not fed gut microflora but had a substantial growth promoting effect on conventionally reared chicks (Harrison and Coates, 1972). Muramatsu *et al.* (1988, 1993) demonstrated that the gut microflora have a positive effect on intestinal protein synthesis and suggested that this was due to the effect of bacterial metabolites released into the lumen. They noted a distinct effect of diet on this, in that a practical diet induced a greater rate of protein synthesis than a purified diet. Increased protein synthesis requires energy which could have, alternatively, been used for growth. In light of these observations, it is possible that the unidentified growth depressing factor found in bacteria-free filtrates in earlier work could have been a metabolite of intestinal bacteria.

2.3.6 Positive Bacterial Impact on the Bird

Most of the work on the positive influence of the GIT bacteria has centred on probiotic use in poultry diets. The word "probiotic" comes from two Greek words meaning "for life". The most common currently accepted definition for probiotic was proposed by Fuller (1989). He states that a probiotic is "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This definition implies that the intestinal microflora can have a

positive influence if the correct types are present. A number of relatively comprehensive reviews have been written on probiotics in animal diets (Barrow, 1992; Fuller, 1989; Jernigan *et al.*, 1985; Sissons, 1989; Stavric and Kornegay, 1995). The general consensus of all of these authors is that the theory behind probiotic use is intriguing and full of possibilities but that until the modes of action are better understood, research will continue to provide conflicting and inconclusive results.

Areas agreed upon to date with respect to microbial probiotics include some of the selection criteria for evaluating probiotic organisms and products. Among the desirable characteristics of a probiotic is the preference that the organism be indigenous to the host animal's gastrointestinal tract (Havenaar *et al.*, 1992). This has been suggested due to the host and location specific nature of the normal gut microflora in addition to the normal microflora's general beneficial properties.

Some consensus in the research community on suggested modes of action has been reached and these are summarized in a review by Stavric and Kornegay (1995). In order to understand the suggested modes of action it is important to note that in poultry, there are two major potential sites of colonization where somewhat different probiotic effects are elucidated. These two areas are the crop, where the lactobacilli colonize and have a potential impact on nutritional and performance parameters, and the caeca, which are the primary colonization site for a number of pathogens and where probiotic products may prevent or reduce this colonization (Barrow, 1992).

The proposed modes of action include competition by probiotic organisms for adhesion receptors on the gut epithelium; competition for nutrients between probiotic

and other organisms; production of inhibitory or bactericidal substances by probiotic organisms; and, stimulation of the immune system of the host animal. Sissons (1989), in his review, adds that some strains of lactic acid bacteria produce an unknown metabolite which neutralizes an enterotoxin released from some coliform bacteria. He also suggests that probiotics may prevent toxic amine synthesis by pathogenic bacteria.

The strains used in probiotic preparations are, as mentioned earlier, normally of intestinal origin. Most are lactic acid bacteria in single or multiple strain cultures. Stavric and Kornegay (1995) state that for commercial probiotics world-wide, the most commonly utilized genera of bacteria are *Bifidobacterium, Lactobacillus, Enterococcus, Bacillus, Bacteroides, Pediococcus, Leuconostoc,* and *Propionibacterium.* The reasons why *Lactobacillus* and *Pediococcus* are good candidates for probiotic preparations are outlined in a review by Juven *et al.* (1991). These genera are able to colonize the gastrointestinal tract of poultry and they also produce a number of compounds believed to have either antagonistic or bactericidal effects. These compounds include lactic acid, acetic acid (and other volatile fatty acids), hydrogen peroxide, and compounds called bacteriocins. Bacteriocins are defined as compounds made by bacteria that have a biologically active protein moiety and a bactericidal action (Juven *et al.*, 1991). Some bacteriocins are very similar to antibiotic compounds of bacterial origin making them of considerable interest for probiotic purposes.

All of the recent reviews on microbial probiotics (Jernigan *et al.*, 1985; Fuller, 1989; Sissons, 1989; Barrow, 1992; Stavric and Kornegay, 1995) summarize reports showing both positive and non-existent or non-significant effects of probiotics on

poultry performance parameters. Effects of probiotics on pathogen control are generally more positive and are usually discussed separately since the effect is in the caeca versus the crop. The major comment of all authors is that field trials with probiotics tend to show variable results.

An evaluation of a number of more recent studies indicates that although the research is generally focussed on more specific effects than performance parameters, the results are still variable. For example, Mohan *et al.* (1995) supplemented the diets of layers with a 5-strain commercial probiotic and cited both a decreased incidence of thin-shelled eggs and a decreased serum cholesterol level in layers ($P<0.05$). Similar work with broilers resulted in a significant reduction in serum cholesterol level in probiotic fed birds but with no significant performance effects after six weeks of growth (Mohan *et al.*, 1996). Owings *et al.* (1990) fed a diet supplemented with *E. faecium* strain M-74 as a probiotic to broilers and compared this to antibiotic or probiotic plus antibiotic treatments. In this trial the basal diet and the diet with just the probiotic resulted in better feed efficiency than the antibiotic or the probiotic plus antibiotic diets. Interestingly, the antibiotic diet resulted in the worst performance. The caeca were shown to be the area of the digestive tract with the highest level of *E. faecium* colonization, a parameter that was also highest for the basal and probiotic supplemented diets versus the antibiotic and antibiotic plus probiotic diets (Owings *et al.*, 1990).

A substantial volume of research on competitive exclusion (or pathogen control) by probiotics and the overall mechanisms involved was reviewed by Fischer (1999). To summarize some of the salient points, the work of Stavric *et al.* (1992) has shown that

undefined cultures with 28-50 strains of bacteria are generally highly effective against *Salmonella typhimurium* colonization when compared with the limited effectiveness or complete lack thereof seen with pure or defined cultures with a small number of strains of bacteria. The use of continuous flow cultures of competitive exclusion organisms was described in the mid-nineties in a number of studies (Nisbet *et al.*, 1993; Corrier *et al.*, 1995; Hume *et al.*, 1995) where the bactericidal effect of undissociated VFAs, particularly propionic acid, on caecal pathogens was demonstrated. Other groups have also commercialized defined (Palmu and Camelin, 1997; Schneitz and Hakkinen, 1998) and undefined (Kelly *et al.*, 1999) cultures that are effective against *Salmonella* and *Clostridium* (Hofacre *et al.*, 1998).

2.3.7 Bacterial Methodology Issues

2.3.7.1 Concerns with Traditional Culturing Methods

Since the bacteria of interest in the GIT are largely anaerobic, the difficulties with isolating and culturing the nearly 400 genera are many.The anaerobic techniques of Hungate (1950) are used but are time-consuming and require experience and care in their execution (Amann *et al.*, 1995). Even with the use of strict anaerobic techniques only an estimated 10% of the GIT bacteria of the chicken have been isolated and cultured (Apajalahti and Bedford, 2000). To effectively culture bacteria, selective media have to be developed and the biochemical properties of each genus and its preferred substrates need to be determined through a series of tests. It has been stated by Apajalahti and Bedford (2000) that the reliance of bacteria growing in complex communities, such as

the GIT, upon growth factors provided by other bacteria and upon secretions from host tissues limits the effectiveness of culturing community members under laboratory conditions.

While these techniques are still in use and are very effective at defining minority populations of pathogens in microbiology (Apajalahti and Bedford, 2000), newer techniques permitting the more definitive identification of bacterial genera are becoming more and more available. The use of 16S ribosomal RNA techniques in combination with in situ hybridization permits relatively rapid and precise identification of bacteria at the species level in labs equipped for molecular analysis (Amann *et al.*, 1995; Bryant, 1997). Sub-species or species of GI bacteria have been shown to change dramatically in response to changes in dietary substrate (Bedford and Apajalahti, 2001). These techniques have been effective in identifying phylogenetic differences not possible using traditional culturing methods (Amann *et al.*, 1995; Langendijk *et al.*, 1995; Snel *et al.*, 1995).

For more general community analysis, a procedure involving percent base composition of the bacterial DNA may be used (Apajalahti *et al.*, 1998). This procedure relies on the adherence of certain groups of bacteria to specific % guanine and cytosine (%G+C) ratios in their DNA. The bacteria need to be purified from the intestinal samples and subsequently analysed for % G+C composition using a series of cesium chloride centrifugations. The procedure, while extremely useful for monitoring dietary effects on bacterial communities in the GIT, is very expensive and only conducted on intestinal samples in one or two corporate laboratories.

2.3.7.2 Volatile Fatty Acid Levels as a Measure of Bacterial Activity

While the procedures for precise identification of bacterial genera and species are either complicated, costly, or require specialized training and equipment, there are other ways of evaluating overall shifts in bacterial activity. One such method is a measure of the end products of bacterial fermentation. Since anaerobic bacteria ferment carbohydrate substrates largely to volatile fatty acids, measuring VFA levels in digesta has been used as a means of quantifying changes in bacterial activity in the GIT of poultry (Corrier et al, 1990a; Choct *et al.*, 1995, 1996, 1999; Vahjen *et al.*, 1998, Yasar and Forbes, 1999; Kocher *et al.*, 2000). Numerous methods exist for VFA analysis in intestinal samples involving processes from steam distillation (Choct *et al.*, 1996) to simple preparation of digesta supernatant with an internal standard and meta-phosphoric acid (Soita, 2001). Most involve the use of gas chromatography to measure VFA content in the samples, regardless of preparation techniques.

2.4 Gastrointestinal Tract Structure and Function

The avian GIT is somewhat unique with its pregastric compartment, the crop, an expanded portion of the esophagus, which serves as a storage area and an area for some microbial fermentation. The stomach is divided into a secretory, glandular stomach, the proventriculus, and a large muscular stomach for mechanical processing, the gizzard.

The sections of the avian GIT of most interest in complex carbohydrate digestion are post-gastric. The long, tubular small intestine has a high absorptive capacity with digestive enzymes (amylase, lipase, trypsin, chymotrypsin, dipeptidases,

aminopeptidases and carboxypeptidases) as well as buffering compounds in aqueous solution from the pancreas being secreted in the duodenum (Duke, 1993). Bile salts are also secreted into the duodenum for the emulsification of fats prior to their digestion. These bile salts are normally reabsorbed in the terminal ileum along with the breakdown products of endogenous proteins (Duke, 1993). Microbial populations are lower in the duodenum and gradually increase in number and proportion of anaerobes distally toward the terminal ileum (Ford, 1974; Salinitro *et al.*, 1978).

2.4.1 Hindgut Digestion in the Chicken

While the duodenum and jejunum are the major sites of nutrient digestion and absorption in poultry (Hurwitz *et al.*, 1979; Riesenfeld *et al.*, 1980), the ileal contents still have a relatively high dry matter content (16-20%) due to undigested materials (Bedford *et al.*, 1991). The residual carbohydrates in the ileum are largely indigestible fibre although these complexes of cell wall material usually include protein (Mares and Stone, 1973; Bacic and Stone, 1981; Fengler and Marquardt, 1988b) bound within them. In corn diets the residual ileal contents include starch resistant to hydrolysis by the bird's enzymes (Brown, 1996). Starch digestibility in the corn-fed broiler although assumed to be high because of fecal digestibility values, has, in fact, been shown to be only 85% in the ileum (Noy and Sklan, 1995). The high rate of passage of digesta in the chicken, generally from 2-4 hours (Mateos *et al.*, 1982; van der Klis and van Voorst, 1993), means that digestive enzymes only have a minimal amount of time to act on their substrates. Anything that hinders their ability to access the substrates is likely to

substantially decrease nutrient digestibility (Bedford and Schulze, 1998). Dietary fibre or NSP that do not get digested by the bird remain in the GIT and are present in the ileum where they can be fermented by the resident microflora.

2.4.1.1 Basic Ileal Morphology and Function in Carbohydrate Digestion

The structure of the ileum is similar to the jejunum and, in fact, some researchers do not separate the two, but prefer to call the region proximal to the Meckel's Diverticulum the upper ileum and the region distal to this, the lower ileum (Duke, 1993). In this document, these two regions will be termed the jejunum and the ileum, respectively, as described by Moran (1982). There is little known about ileal motility in poultry, although both peristalsis and segmenting contractions have been noted and characterized in the turkey (Duke, 1993). The structure of the intestinal layers in poultry is similar to swine but different musculature is emphasized, demonstrating the differences in digestive function between the species. The chicken has a well-developed luminal surface but a poorly developed muscularis mucosa and lamina propria, relative to the pig (Moran, 1982). Poultry have a poorly developed lymphoid system and submucosa but a very well-developed muscle layer which indicates reliance on physical activity in the small intestine for motility and mixing of feed with digestive enzymes (Moran, 1982). The villi in the chicken small intestine are leaf-shaped and decrease in length from jejunum to ileum, but increase in length with age of the bird (Moran, 1982). Microvillus length also decreases from jejunum to ileum but microvillus length actually decreases in the ileum with age of the bird and density increases (Ferrer *et al.*, 1995).

Digestion and absorption of carbohydrates occurs in the enterocyte brush border which involves the glycocalyx, the microvilli and the underlying terminal web of cell walls, tight junctions and cytoplasmic filaments. Pancreatic enzymes start digestion but the products that result are still too large to be absorbed, so digestion is completed by the enzymes associated with the microvilli (such as sucrase and maltase) (Moran, 1982). Goblet cells increase in number toward the distal end of the ileum and secrete mucin (made of water-soluble glycoproteins) to aid in lubrication of the increasingly fibrous digesta as it passes through the intestine (Moran, 1982). Mucin contributes to the "unstirred water layer" which limits nutrient absorption.

The carbohydrates that remain in the ileum of the bird are still readily absorbed. The ileal epithelium is capable of transporting glucose and other monosaccharides, but different monosaccharides are absorbed from the GIT at different rates. This has been measured both by ileal and excreta digestibility or AMEn of different monosaccharides (Longstaff *et al.*, 1988; Schutte *et al.*, 1992) as well as by ^{14}C recovery in CO_2, excreta, and body tissues after supplementation with ^{14}C-labelled monosaccharides and cell wall substrates (Savory, 1992a; 1992b). Glucose is absorbed well from all SI segments and its rate of passive absorption is concentration dependent (Riesenfeld *et al.*, 1980). Amat *et al.* (1996) determined that the jejunum is the segment of the chicken small intestine best suited for Na^+-mediated uptake of hexoses while in the duodenum these sugars are largely absorbed by passive diffusion. Hexoses are absorbed from all segments of the small intestine by both mechanisms, although to a much lesser extent in the ileum (Amat *et al.*, 1996).

Longstaff *et al.* (1988) determined that the relative AMEn values provided to chicks by certain monosaccharides known to be poorly utilized were in the following order relative to glucose:

glucose>xylose>arabinose>galacturonic acid>glucuronic acid

The dietary inclusion rate of the pentose sugars, however, had a significant effect on their derived AMEn values with higher levels resulting in lower derived AMEn (Schutte, 1990) as well as serious negative effects on production parameters (Wagh and Waibel, 1967a; Baker, 1977; Longstaff *et al.*, 1988; Schutte, 1990; Schutte *et al.*, 1992). The effect was more pronounced for arabinose than for xylose. Bogner (1961), working with embryos and very young chicks, found that by 3 d of age, absorption of glucose was as fast as in 14 d chicks, despite all sugars having equally slow absorption rates in the embryo or immediately after hatch. Once the system for preferential absorption of glucose was established at 3 d, the rates for pentose absorption, relative to glucose, were 79.7% for xylose and only 45.6% for arabinose. Other experiments confirmed that xylose is absorbed faster than arabinose using both subcutaneous injection of the monosaccharides (Wagh and Waibel, 1967a) and crop infusion (Wagh and Waibel, 1967b). The latter study demonstrated that arabinose is retained in the SI much longer than xylose and is absorbed much more slowly. Longstaff *et al.* (1988) determined that the digestibilities of monosaccharides followed the same order as AMEn with respect to the relationship between glucose, xylose and arabinose. These observations were confirmed by Schutte *et al.* (1991) and Savory (1992b) and were similar for pigs (Yule and Fuller, 1992).

2.4.1.2 Specific Effects of NSP on the Ileum

The consumption of elevated levels of dietary NSP has a number of effects in the ileum and many of these were highlighted in Section 2.1.3.1. Langhout *et al.* (1999) demonstrated that the viscosity generating potential of the NSP makes a big difference in the impact of the NSP on both microflora and ileal morphology. These researchers noted that a high-methylated citrus pectin significantly increased both the number of goblet cells per 100 villi and the activity of sucrase-isomaltase in the ileum. Low-methylated citrus pectin did not have a significant effect on either of these parameters (Langhout *et al.*, 1999).

The monosaccharide content of NSP also has implications in ileal function. Should the NSP be broken down to its constituent monosaccharides, absorption in the small intestine would be slow, since arabinose and xylose are slowly absorbed relative to glucose, and, as a result, some of the xylose and even more of the arabinose would end up in the distal ileum as accessible microbial substrate. Savory (1992a) demonstrated jejunal absorption of glucose to be 1.9 times faster than xylose. Further possible evidence for this potential is seen in the work of Schutte *et al.* (1992) who showed that the utilization of xylose was only 20% that of glucose and that of arabinose was 0% of glucose. These observations seem to contradict earlier results where Schutte *et al.* (1991) determined the ileal digestibilities of xylose and arabinose to be 99.8% and 74.6-95.5% (depending upon dietary inclusion level), respectively. The 1992 study, however, determined utilization by assuming that any pentose sugars not deposited in tissue or excreted were utilized. This does not take into consideration the fermentation of the

pentose sugars by the microflora present in the GIT. It is likely that ileal digestibility values were confounded by microbial fermentation of the monosaccharides in the ileum versus actual utilization by the bird. Suprisingly, the fecal digestibilities determined by Longstaff *et al.* (1988) were not very different (97.9% for xylose and 77.9% for arabinose) from Schutte *et al.*'s (1991) ileal values.

2.4.1.3 Caecal Morphology and Function in Carbohydrate Digestion

The caeca are of particular interest for investigations of gut-microbe interactions in carbohydrate digestion. The avian caeca are outpocketings of the digestive tract which are blind tubular sacs directed backwards along the terminal portion of the ileum and connected to it by mesenteric tissue. The caeca are usually found as a pair, although substantial interspecies variation exists in size, shape and in number with some species of birds having no caeca, some with vestigial caeca and some with just one caecum (McLelland, 1989).

The caeca arise at the ileo-caecal-colonic junction (the junction between the small and large intestine). A muscular ring of tissue projects into the lumen of the intestine just anterior to the caecal openings, which are narrow and lined with villi. It is believed that this structure is related to the filtering of material during caecal filling (Strong *et al.*, 1989; Bjornhag, 1989). Antiperistaltic movements of the colon are responsible for caecal filling and for the entry of urine into the caeca. This retrograde transport of urine into the caeca, through the filtering system at the entrance, is theoretically a means of only permitting smaller particles of digesta with large surface

area relative to volume into the caeca so that the bacteria only have access to readily fermentable materials (Bjornhag, 1989; Moss, 1989; Remington, 1989). The larger, less digestible materials are separated out and rapidly excreted. The short tract of birds necessitates the maintenance of a mechanism for separating the poorly fermentable from easily fermentable digesta to permit the bird to take advantage of high levels of food intake and rapid digesta passage rates.

The caeca can be divided into three regions based on the morphology of the epithelium. The morphology of the epithelium of the proximal caeca is very similar to that of the jejunum with well-developed villi, long microvilli, and large numbers of goblet cells. The middle and distal regions of the caeca have patterns of folds and poorly developed villi and microvilli. All factors point to the proximal region as the major site of absorption in the caeca with the suggestion that the middle and distal segments have a role in storage and fermentation (Sudo and Duke, 1980; Dantzer, 1989; Strong et. al., 1989; Ferrer et. al., 1991). In fact much work in the eighties divulged the existence of a Na^+-dependent, phloridzin-sensitive hexose sugar transport mechanism in the proximal cecum with transport kinetics and capacities similar to those in the jejunum (Ferrer *et al.*, 1986; Planas *et al.*, 1986; Vinardell *et al.*, 1986). While jejunal hexose transport rates decline significantly between 2 and 21 d of age (Shehata *et al.*, 1981) this age-related change has not been studied in the caecum. In the caecum, however, there is a distinct decrease in hexose transport capability from the proximal to the distal end of the caecum which corresponds to a reduction in apical cell surface area due to reduced microvilli length (Planas *et al.*, 1987).

While caecal filling has been shown to be continuous (Savory and Knox, 1991), the left and right caeca are voided 1-2 times per day in the domestic fowl (Thomas and Skadhauge, 1988) in a simultaneous peristaltic rush starting at the distal end of each caecum and continuing through the colon and cloaca. Caecal excreta resulting from evacuation are distinct from regular excreta in their dark colour and paste-like appearance. Regular caecal contractions aside from those associated with caecal evacuation, however, are not coordinated between left and right caeca and operate in both posterior and anterior directions (Duke et. al., 1983). This regular contractile activity facilitates mixing of the digesta and retrograde urine throughout their retention time in the caeca.

Volatile fatty acids, in particular acetate, propionate, and butyrate, are the end products of bacterial fermentation of carbohydrate (and protein) substrates. If fed to birds, all VFAs are completely absorbed before the ileo-caecal-colonic junction. High levels of VFAs in poultry excreta, however, are indicative of microbial production in the hindgut (Bolton and Dewar, 1964). Further support of this is shown by a comparison of VFA levels in the droppings of caecectomized (36.8 mmol/kg) and non-caecectomized (91.8 mmol/kg) fowl (Annison et. al., 1968).

VFA concentrations in poultry caeca were found by Savory and Knox (1991) to be in the proportions 72:22:16 of acetate, propionate, and butyrate, respectively. Annison *et al.* (1968) suggest that VFA are absorbed from the caeca and metabolized in the liver since the portal blood supply of poultry was shown to contain all of the VFAs found in the caeca but the peripheral blood supply was only shown to have acetic and

formic acids. A comparison between conventional and germ-free birds indicated that peripheral blood supply levels of acetic acid were similar between the two types of birds. The researchers (Annison et. al., 1968) felt that this showed that acetic acid was largely of endogenous origin rather than of microbial origin. The proportion of acetate absorbed from the digestive tract (primarily the caeca) was, however, reported to be approximately 25% of total acetate production in the fed bird and was said to account for 3% of the total daily energy requirements of the fowl. Gasaway (1976a, 1976b) showed that the metabolizable energy available from VFA averaged 7.1 kcal/d which was equivalent to about 7% of the daily free living energy requirement of Rock Ptarmigan and 5.7 kcal/d or 3.8% of daily free living energy requirement of Willow Ptarmigan. Similar calculations do not appear to have been made for domestic poultry since those made by Annison *et al.* (1968), because there has been little evidence that poultry obtain energy from caecal VFA absorption. In the one recent exception, Jørgensen *et al.* (1996) calculated that fermentation of NSP from high fibre pea diets by the GIT microbes of the chicken could contribute approximately 3-4% of the intake of ME. While Carré *et al.* (1995) speculate on the contribution of NSP fermentation to ME in chickens, they caution that the process is only 50% as efficient as the provision of energy from glucose in growing chickens.

2.4.1.4 Specific Effects of NSP on the Caeca

Little has been published on the effect of NSP on caecal morphology of birds. The research published on the effect of pentose sugars on the caeca demonstrates that

feeding of L-arabinose markedly increases caecal weight and length while D-xylose has a less dramatic effect (Longstaff *et al.*, 1988; Schutte, 1990; Schutte *et al.*, 1992). In terms of actual physical entry of larger polymer NSP into the caeca, very little to no research has been published in this area. Many of the bacteria present in the caeca are capable of fermenting NSP. Although most strains were found by Mead (1989) to be able to grow on arabinoxylan, most of the early work on caecal fermentation was to determine cellulose digestibility and did not focus at all on hemicellulose. This work was done first in the ptarmigan (Gasaway 1976a,b,c), the turkey (Duke *et al.*, 1984) and then the fowl (Savory and Knox, 1991; Savory, 1992a). All of these researchers used radio-labelled cellulose (^{14}C) or glucose and xylose (Savory and Knox, 1991; Savory, 1992a) fed to or introduced into the GIT of the birds, and measured $^{14}CO_2$ output or levels of ^{14}C in plasma and tissue to determine utilization.

In terms of the effect of NSP on caecal function, the issue of whether or not the microflora of the avian caeca is able to digest cellulose has been a topic of discussion in the literature for a number of years. Mead (1989) found no evidence of cellulase activity in the caecal microflora. Other researchers, however, have conducted a variety of trials indicating that cellulose digestion does occur in the caeca. A number of researchers have provided indirect evidence that wild birds such as certain species of ptarmigan and grouse have the capability to digest cellulose in the caeca (Gasaway, 1976c; Moss, 1989; Redig, 1989; Remington, 1989). In domestic birds, however, this ability has only been demonstrated with birds preconditioned to high fibre diets (Duke et. al., 1984; Redig, 1989; Savory, 1992b). This would support the proposal by Moss (1989) that

galliform birds may have digestive tracts which operate in one of two states: a low fibre mode where bulk does not limit intake and the sizes of the caeca and small intestine do not vary with intake, and a high fibre mode where bulk does limit intake and therefore the sizes of the caeca and small intestine do increase past a threshold intake level and the caeca become important to energy metabolism.

The practical determination of the effect of dietary arabinoxylans on the caeca is difficult since most of the literature in poultry focuses on the impact of these NSP on ileal physicochemical characteristics. While we know that changes in the ileum will affect digesta flow and therefore will likely have an impact on substrate entry into the caeca, little has been published in this area. The size and the solubility of the arabinoxylans at the terminal ileum are likely the most important characteristics to have an impact on whether the NSP gain entry to the caeca. Much of the literature states that only small, water soluble, readily fermentable particles enter the caeca (Gasaway *et al.*, 1975; Bjornhag, 1989; Moss, 1989; Remington, 1989). The small, soluble arabinoxylans are, therefore, likely to enter the caeca, while the larger, insoluble ones may not enter. Some support of this concept is seen in work with high viscosity diets with added arabinoxylans fed with or without endoxylanase supplementation. The experiments of Choct *et al.* (1995, 1996, 1999) clearly demonstrate increased caecal fermentation occuring with enzyme addition. It is logically assumed that the enzyme increases the proportion of small, soluble arabinoxylans that can enter the caeca. Without enzyme supplementation, the ileal digesta viscosity is so high that entry into the caeca may well be impeded by viscous conglomerations of digesta.

The increase in proliferation of bifidobacteria in the caecum with fructooligosaccharide (FOS) administration through the drinking water has been demonstrated in rats and mice (Howard *et al.*, 1995). A trophic effect of FOS on the colonic mucosa was also observed. Significantly increased crypt cell depth in the caeca was observed with similar administration of xylooligosaccharides to rats and mice in the same study. Another study evaluating the feeding of mannan oligosaccharides (MOS) to turkey poults (Savage *et al.*, 1997) demonstrated increased goblet cell numbers and villus width but decreased crypt depth. This study also was able to demonstrate significantly ($P<0.04$) improved weight gain and feed efficiency with 0.1% addition of MOS. It was thought that the improved performance was related to changes in the bacterial flora. It is suggested that mannose exerts an antibacterial effect by blocking receptor sites at the gut epithelial surface (Bailey *et al.*, 1991). Some gut bacteria have mannose-specific adherence appendages and hence competition for binding sites at the intestinal brush border may occur (Sissons, 1989).

Alternative types of oligosaccharides include kestose oligosaccharides (KOS) produced by the pyrolysis of sucrose and another, easier to produce product, sucrose thermal oligosaccharide caramel (STOC). KOS were shown to have no effect on performance parameters of broilers fed 10% crude kestoses and no effect on total aerobic, coliform, total anaerobes, aerobically enumerated lactobacilli or clostridia in the caeca (Patterson *et al.*, 1997). KOS did, however, increase caecal bifidobacteria 24-fold and anaerobically enumerated lactobacilli 7-fold. STOC were also shown to increase caecal bifidobacteria numbers but this type of OS also reduced caecal aerobes and

coliforms and improved bird performance, particularly under stressful conditions induced by either heat stress or vitamin/mineral deficiencies (Orban *et al.*, 1997).

The use of FOS in pathogen control has also been evaluated. Chambers *et al.* (1997) compared crude FOS, refined FOS and some lactose derivatives and found that *Salmonella typhimurium* counts were lowest for birds fed refined FOS. It was also noted that pH and caecal density were both lower for birds fed FOS or lactose derivatives than for the control birds however no consistent effect of pH or caecal density on *Salmonella* numbers was observed. Stavric and Kornegay (1995) observed from their review of the literature that FOS have a better effect in poultry when fed with a probiotic, particularly with competitive exclusion cultures. This suggestion is supported by the work of Bailey *et al.* (1991) where *Salmonella* numbers were decreased 12% (not significant) by feeding FOS, 24% (significant) by feeding a competitive exclusion culture, and 76% by feeding both. This same study showed that when stressed chickens were fed FOS only 25% were colonized versus 92% colonization of stressed controls. It was suggested that perhaps the gut alterations caused by feeding FOS might also decrease the bird's susceptibility to stress.

2.4.1.5 Other Roles of the Caeca that Affect their Function

Of the functions attributed to the caeca that are not related to carbohydrate digestion, the area of protein digestion or nitrogen metabolism is one of the most extensively studied areas. In birds, uric acid is the end product of nitrogen metabolism and is present as small spheres that can readily pass through the duct system of the

kidneys. The retrograde peristalsis of the colon, which causes refluxing of urine into the caeca, provides an effective mechanism for reclamation of some of the carbon and nitrogen from urine. Uric acid is rapidly broken down by caecal microbes and the end products include ammonia, acetate, CO_2, glycine, formate, and propionate (Braun and Campbell, 1989; Karasawa, 1989).

The ammonia from uric acid decomposition is incorporated into α-ketoglutarate to form glutamic acid but uptake of glutamate by the epithelium has not been shown (Braun and Campbell, 1989). Uptake of a number of other amino acids by the caecal epithelium has been demonstrated including lysine, leucine, proline, aspartate (Obst and Diamond, 1989), phenylalanine and valine (Moretó and Planas, 1989) indicating that investigation into the potential transport of glutamine is warranted.

Although ammonia is produced from uric acid by caecal bacteria (Barnes and Impey, 1974; Mead and Adams, 1975), uric acid is not a required substrate for caecal bacteria (Barnes and Impey, 1974). This is evident in the fact that ammonia utilized by the caecal microflora can also be derived from microbial breakdown of L-arginine, glutamine amide, glutamic acid, glycine and alanine (Karasawa, 1989). Therefore, the bacteria can obtain N from many sources, both endogenous and dietary, for their metabolic activities.

Karasawa (1989) suggests that the ammonia produced by the ureolytic caecal bacteria, and not utilized by other bacteria present, could be a substrate for non-essential amino acid synthesis and may be a pathway by which dietary urea is utilized, ultimately, for protein synthesis in poultry. In fact, Karasawa and Maeda (1995) demonstrated that

despite the degradation of urea to ammonia by the caecal bacteria, the N is mostly absorbed from the caeca as protein, urea and other amino acids, rather than directly as ammonia. Caecal nitrogen processing may be most important in protein-depleted chickens where the caeca would play a role in conserving short supplies of nitrogen by recycling waste nitrogen in the body. Removal of the caeca either by ligation and washing out of the contents or by caecectomy, however, actually results in an increase in nitrogen retention and utilization in both adult and growing chickens (Son and Karasawa, 2000; Son *et al.*, 2000).

The caeca have been shown to be the primary sites of water and electrolyte resorption in birds (Thomas and Skadhauge, 1988; Chaplin, 1989; Goldstein, 1989; Thomas and Skadhauge, 1989). Caecectomy significantly increases excreta moisture content and GIT passage rate in growing chicks (Son *et al.*, 2000). Despite this fact, caecectomized birds adapt well to their loss and after 3 weeks show no difference in water intake or excreta moisture levels from control birds (Chaplin, 1989). Electrolyte and water absorption occurs via a sodium dependent active transport system that is able to account for virtually all of the net water uptake from the hindgut (Thomas and Skadhauge, 1989; Grubb, 1991). Again, adaptation to normal conditions within 10-15 days of caecectomy provides evidence that the bird has considerable reserve capacity for these functions elsewhere in the renal-gastrointestinal system (Thomas and Skadhauge, 1989). It is suggested that these particular functions of the caeca are much more crucial in dehydrated or heat-stressed birds.

It has been known for some time that caecal bacteria synthesize a number of B-

vitamins and that the levels of these B-vitamins in caecal contents can be quite substantial. McNab (1973) in a review of the literature, however, reported that the bird was unlikely to derive any benefit from this synthesis unless coprophagy was practised. Vitamin synthesis and absorption in the caecum was not even discussed at a more recent symposium on the function of the avian caecum (Braun and Duke, 1989).

The role of the caeca in immune function is uncertain, however a large body of research has focussed on the role of the caeca in pathogen control. The recent emphasis has been on the feeding of lactose as a means of lowering caecal pH in chicks inoculated with caecal microflora in order to increase the concentrations of bacteriostatic volatile fatty acids in the caeca. This has been shown to decrease caecal colonization by pathogenic strains of bacteria (Morishita *et al.*, 1982; Corrier et. al., 1990b; Hume et. al., 1992; Hume et. al., 1995).

2.5 Bacterial Fermentation of Non-starch Polysaccharides in the Gastrointestinal Tract

In addition to age or developmental changes in microflora of the GIT, the second most influential factor, practically speaking, is diet. Caecal microflora have seemingly been studied more comprehensively than SI flora and appear to be less affected by minor dietary changes, such as varying protein levels, than by more basic changes such as the dietary fibre or NSP level of ingredients used (Mead, 1997). It has been said that the chemical composition of the digesta is one of the major determinants of the makeup of the GIT bacterial community (Apajalahti and Bedford, 2000). Naturally, this provision

of substrate has a significant impact on bacterial fermentation in the GIT.

2.5.1 Fermentation of Non-starch Polysaccharides

As a site of fermentation, conditions in the avian caeca are ideal for bacterial proliferation. The substrate is liquid, the environment is anaerobic, the pH is between 6.5 and 7.5 and the site is evacuated on a regular basis. Over 200 different strains of bacteria have been isolated from the GIT of the chicken (Apajalahti and Bedford, 2000), the principal organisms of which were discussed previously. The obligate anaerobes, as mentioned, are found at levels of 10^{11}/g with the facultative anaerobes appearing at much lower levels (Mead, 1989). Almost all of the bacteria present utilize glucose as a substrate, while a smaller proportion is able to utilize lactate. Most strains were found by Mead (1989) to be able to grow on arabinoxylan. This is supported by the work of Longstaff *et al.* (1988), Savory (1992c) and Schutte *et al.* (1992) all of whom demonstrated, using either relative caecal weights or recovery of radio-label in caecal contents, significant caecal fermentation of the monosaccharides arabinose and xylose if included at high enough levels.

Depending upon GIT conditions and age of the bird, dietary NSP may be broken down by bacterial enzymes when it reaches the terminal ileum and some monosaccharides are, therefore, likely to be present. Most monosaccharides perfused into the fowl are absorbed before reaching the caeca but arabinose, as discussed previously, has a relatively low rate of absorption and hence is still present in the fluid entering the caeca. When xylose was perfused directly into GI segments, it was shown

to be absorbed faster in the caecum than in the small intestine (Savory, 1992a). Although much is known about the potential for absorption of pentose sugars, little has been published on the actual amount of xylose or arabinose from dietary NSP present at the terminal ileum and available either for absorption by the bird or fermentation by the microflora. What is published shows that intact or partially degraded cell wall material is still found at the end of the digestive tract of poultry (Bedford and Autio, 1996). The quantity present and the degree of degradation of this cell wall material that takes place depends upon the composition of the microflora in the GIT and upon the composition of the diet being fed to the bird (Bedford, 1996b). Little is known about what proportion of NSP gains entry into the caeca or what the characteristics of that NSP are. As outlined previously, it is likely that the NSP has to be relatively small and largely soluble in order to enter the caeca.

2.5.2 Fermentation of Oligosaccharides

Partially degraded NSP may be depolymerized enough to become oligosaccharides. These are preferential substrates for some intestinal bacteria due to the less complex structure and, therefore, the ease with which they can be degraded by the bacteria. The use of such complex carbohydrates from soluble fibre as probiotic-like products has gained considerable interest in recent years. The products considered of use commercially in encouraging the preferential growth of beneficial bacteria in the hindgut, or exerting a "prebiotic" effect, are oligosaccharides, particularly fructooligosaccharides which are linear chains of ß-D-fructofuranose units linked by

glycosidic bonds (Monsan and Paul, 1995). These products, if added in the diet, resist digestion in the foregut of the host animal and are able to reach the colon or caecum where they can interact with the microflora of the hindgut and act as a substrate for bacterial growth (Roberfroid, 1993). Other oligosaccharides that may be of value as feed additives include α-glucooligosaccharides (isomaltooligosaccharides), α-galactooligosaccharides, ß-glycooligosaccharides, and ß-xylooligosaccharides. All of these products vary somewhat in the types of sugars present and the linkages between the sugar moieties (Monsan and Paul, 1995) and all of them are potential breakdown products of the NSP found in feed ingredients.

The suggested modes of action of the oligosaccharides (OS) include specific substrate feeding, whereby some beneficial gut microbes, particularly the genus *Bifidobacterium,* can specifically use OS whereas the pathogenic organisms cannot (Kohmoto *et al.*, 1991; Roberfroid, 1993; Howard *et al.*, 1995; Monsan and Paul, 1995). It is also suggested that OS feeding can: reduce the amounts of "putrefactive" degradation products of certain amino acids in faeces and urine; induce enzyme production by intestinal bacteria, thereby increasing the hydrolysis of insoluble carbohydrate polymers; interact with protein receptors on microbial cells and brush border epithelial cells, thereby interfering with pathogenic binding to these receptors; and influence secretion of immunoglobulins, activate the immune response or preserve systemic immunity by preventing bacterial translocation from the gut (Monsan and Paul, 1995). Very little research is available supporting these latter suggested mechanisms.

2.5.3 Fate of the End Products of Non-starch Polysaccharide Fermentation

Gasaway (1976a, 1976b) calculated absorption rates of VFA by measuring disappearance from caecal contents of ptarmigan and found the absorption of butyrate to be fastest followed by propionate and then acetate. This same order of absorption of VFAs occurs in most mammals studied (McBee, 1989). Butyrate was also shown to yield the greatest metabolizable energy, followed by propionate and acetate (Gasaway, 1976b) but this was affected by time of caecal emptying. Energy produced from caecal fermentation in ptarmigan was shown to be highest just prior to caecal emptying. The contribution to ME from caecal fermentation was calculated, in this experiment, by multiplying the moles of VFA produced in the caeca per day by the respective caloric value for the heat of combustion of each VFA.

In terms of the kinetics of absorption of VFA in the chicken caeca, Sudo and Duke (1980) showed that propionate and butyrate were absorbed at the same rate from the caeca and the small intestine. Acetate was actually absorbed faster in the caeca. The researchers felt that this was consistent with the kinetics of passive transport of VFA. They were somewhat surprised that VFA were absorbed as well in the caeca as the small intestine because the small intestine has more mucosal surface area than the caeca. This would imply that the caeca are more permeable to VFA than the small intestine and hence the caeca may have a unique absorption system which disregards the normal kinetics whereby VFAs are absorbed at rates proportional to chain length. The concept of differential rates of nutrient transport is partially supported by the work of Savory (1992a) in which it is shown that sugars are absorbed at different rates from the caeca

versus the jejunum.

Most of the literature indicates a minimal contribution of the VFA produced in the caeca to the nutrition of the domestic fowl under normal conditions. One further role of the VFA has been elucidated, however, which involves VFA as a substrate for transepithelial ion transport. This relates to the role of the avian caeca in water and electrolyte balance. Glucose normally acts as an energy substrate for sodium ion transport across epithelial membranes. Thomas and Skadhauge (1988), however, demonstrated that sodium flux operates against a prevailing electrochemical potential difference and that acetate can stimulate active Na transport. Acetate and butyrate were shown to be equally effective in stimulating sodium transport across a membrane *in vitro* regardless of which side of the membrane they were placed on (Grubb, 1991). Evidence points to the utilization of the VFAs as substrates for ion transport rather than stimulants thereof. Propionate did not directly inhibit sodium ion transport across the membrane when administered, but it failed to serve as an energy substrate.

Goldstein (1989) proposed a number of possible fates of the VFA produced by caecal microflora. He suggested that VFAs could be metabolized by caecal tissues and thus contribute the energy necessary for active transport of other solutes thereby enhancing the gradient for movement of sodium into the cell. This mechanism has been demonstrated in mammals but has not yet been shown in birds. Goldstein (1989) also suggested that transport pathways for ions and VFAs may interact through secretion of hydrogen ions. VFAs are presumed to be passively absorbed through caecal epithelial cell membranes and must be in their protonated form to do so. This means that the

hydrogen ions necessary for protonation might come from hydration of CO_2 produced by microbial fermentation or from secretion of hydrogen ions into the caecal lumen via the Na^+/H^+ exchange system.

2.6 Effects of Enzyme Supplementation of High NSP Feed Ingredients

Initial enzyme supplementation of poultry feeds was largely done with amylase and protease products. This was logical since the observed digestibility problems were with starch and protein. These enzyme products yielded somewhat variable results and it was soon determined that positive performance results were being observed when the amylase or protease products were impure and were found to have ß-glucanase or pentosanase activity (Chesson, 1987; Campbell and Bedford, 1992). Current commercial enzyme products for use in poultry feeds are often preparations with a range of substrate specificities. Since cereal grains often contain both ß-glucans and pentosans, enzyme preparations with both ß-glucanase and pentosanase (endoxylanase) activity, i.e. multi-enzyme systems, are likely best for optimal NSP breakdown. The ß-glucanases and xylanases used, specific for the viscosity generating NSP of cereal grains, are largely endo-enzymes capable of randomly hydrolysing linkages within a polysaccharide chain thus shortening the chain and reducing its gel-forming properties (Chesson, 1987).

2.6.1 Impact of Enzymes on Bird Performance

It is well established that exogenous NSP enzymes generally yield improvements in bird performance. The magnitude of improvement is dependent upon a number of

factors including the target substrate, the activity or specificity of the enzyme, itself, and the age of the bird being fed (Huyghebaert and Schöner, 1999). Potter *et al.* (1965) demonstrated improvements in the feed efficiency of chicks fed barley diets supplemented with a crude fungal enzyme preparation. An attempt was made by Rotter *et al.* (1989) to correlate broiler chick response to enzyme supplementation of barley diets with barley extract viscosity. This was found to vary with the method of extraction, with the shear force used, and with the cultivar of barley that was fed. Pawlik *et al.* (1990) using the same crude enzyme preparation in rye-fed birds, observed improvements in both weight gain and feed efficiency over unsupplemented, rye-fed birds. Pettersson *et al.* (1990) showed clear improvements in body weight, feed intake and feed conversion ratio of broilers on barley and rye-based diets supplemented with ß-glucanase and arabinoxylanase, respectively. Veldman and Vahl (1994) demonstrated performance improvements in wheat-fed broilers with xylanase supplementation, regardless of wheat type. Performance improvements with enzyme supplementation can be greatly influenced by other dietary factors, particularly type of fat with saturated fats, and lower initial performance parameters, resulting in greater relative improvements with enzyme supplementation (Dänicke *et al.*, 1997b; Langhout *et al.*, 1997). This is logical, since the performance of birds fed highly viscous cereal diets has been shown to be more negatively influenced by saturated versus unsaturated fats (Antoniou *et al.*, 1980; Antoniou and Marquardt, 1982; Ward and Marquardt, 1983).

Much of the improvement in performance relates to reductions in digesta viscosity. While Cowan (1995) points out that reducing viscosity below 10 cps does not

yield additional improvements in performance, Bedford and Morgan (1996) argue that the improvements in performance seen with enzyme supplementation are often most significant after the digesta viscosity effect is no longer significant. The greatest relative improvement in feed conversion ratio across 14 trials was actually seen from 21-42 d of age (Bedford and Morgan, 1996). These researchers speculate on the possible involvement of the established microflora of the older bird in the performance response to enzyme supplementation. Reports of performance improvements with xylanase supplementation of wheat diets continue to be generated (Steenfeldt *et al.*, 1998a).

2.6.2 Digesta Viscosity and Physical GIT Characteristics

Studies continue to demonstrate viscosity reduction in intestinal contents of birds fed ß-glucanase supplemented barley (Hesselmann and Åman, 1986; Villamide *et al.*, 1997) and oat (Campbell *et al.*, 1987) diets and arabinoxylanase supplemented rye (Grootwassink *et al.*, 1989; Pettersson and Åman, 1989; Bedford *et al.*, 1991; Bedford and Classen, 1992) and wheat (Bedford and Classen, 1992; van der Klis, 1993; Cowan, 1995; Morgan and Bedford, 1995; van der Klis *et al.*, 1995; Steenfeldt *et al.*, 1998a) diets. Speculation also continues on whether improved performance resulting from enzyme supplementation is due to a direct effect of viscosity reduction enabling more ready diffusion of substrates, enzymes and other products of digestion, whether the enzymatic degradation of NSP removes a physical barrier between digestive enzymes and plant cell nutrients, or whether NSP breakdown prevents microbial overgrowth in the small intestine thus allowing the bird more access to nutrients in the digesta.

With rye diets, it has been suggested that the direct effect of xylanase supplementation on intestinal viscosity is more important than endogenous enzyme/substrate access (Bedford *et al.*, 1991). These researchers have shown in two experiments (Bedford *et al.*, 1991; Bedford and Classen, 1992) that enzyme supplementation of rye diets decreases the intestinal concentration of a high molecular weight carbohydrate fraction. This carbohydrate fraction (>500 kDa) has been shown to correlate well with the log of intestinal viscosity and this relationship has been shown to be similar for wheat diets suggesting that wheat and rye release high molecular weight carbohydrates with similar viscous properties (Bedford and Classen, 1992). More recently, Bedford and Apajalahti (2001) have demonstrated that enzyme supplementation dramatically increases the concentration of xylo-oligomers in three categories measured (dp<10; dp<100; and dp<500). The relative increase in the polymers of the dp<10 size is greatest, resulting in large amounts of soluble substrate for the resident microflora to break down and utilize.

The grain content of the diet also affects the degree of response seen to enzyme supplementation. In wheat diets, the negative effects of viscosity on unsupplemented diets appeared to be less in diets with 60% wheat versus those with 80% wheat, and, as a result, the positive impact of the enzyme was less in the lower wheat content diets (Steenfeldt *et al.*, 1998a). Digesta viscosity has become a standard for estimating the contribution of viscous NSP to the performance of broiler chickens as well as the contribution of enzyme supplementation to improving this. Gut viscosity, however, is only a useful predictor of animal response to enzyme supplementation in birds fed

specific highly viscous grains supplemented with viscosity-reducing enzymes under set conditions (Choct, 2001).

2.6.3 Impact of Enzymes on Nutrient Absorption

The reduction in intestinal viscosity with appropriate enzyme supplementation has been shown to improve starch, lipid and nitrogen digestibility in both barley (Hesselman and Åman, 1986; Petterson *et al.*, 1990; Rotter *et al.*, 1990; Friesen *et al.*, 1992) and wheat/rye diets (Fengler *et al.*, 1988; Pettersson and Åman, 1989; Pawlik *et al.*, 1990; Pettersson *et al.*, 1990; Friesen *et al.*, 1991; Friesen *et al.*, 1992; Steenfeldt *et al.*, 1998b). Dänicke *et al.* (1997b), working with rye diets, observed an interaction between dietary fat type and response to xylanase supplementation in that tallow-fed birds responded with greater increases in fat digestibilty than soya oil-fed birds. The same observations were made by Langhout *et al.* (1997) while feeding a wheat/rye diet either with soya oil or a blended animal fat supplemented with a different xylanase product.

Both reduced viscosity and a reduction of endogenous amino acid losses were credited for the increased ileal amino acid digestibility of carbohydrase supplemented wheat diets observed by Hew *et al.* (1995). Van der Klis *et al.* (1995) demonstrated a clear negative relationship between digesta viscosity and both dry matter and mineral absorption in the distal jejunum and ileum. Endoxylanase supplementation of the diet reduced digesta viscosity in wheat-based diets and enhanced apparent absorption of Ca, Mg, Na, and K in the jejunum but only improved Mg absorption from the ileum (van der

Klis *et al.*, 1995).

2.6.4 Impact of Enzymes on AMEn of Cereal Grains

Enzyme supplementation of diets high in NSP has also been shown to reduce variability in AMEn (Choct *et al.*, 1995, 1996; Scott *et al.*, 1998a, b, c, 1999) in addition to increasing AMEn of the cereal grain used (Potter *et al.*, 1965; Rotter *et al.*, 1990; Friesen *et al.*, 1991; Huyghebaert *et al.*, 1995; Steenfeldt *et al.*, 1998b; Huyghebaert and Schöner, 1999). Ideally, this positive effect could lead to prediction equations for AMEn of cereal grains based on enzyme supplementation. The increases in AMEn as a result of enzyme supplementation, however, are not consistent figures. Studies have shown the AMEn to be increased more for high viscosity grains than for low viscosity grains of the same species (Annison, 1993; Villamide *et al.*, 1997; Scott *et al.*, 1998c). The variability in NSP content between different feed ingredients means that enzymes of differing activities elicit different responses. This has been shown in both protein ingredients (Annison *et al.*, 1995; Annison *et al.*, 1996; Huyghebart *et al.*, 1995; Hughes *et al.*, 2000; Kocher *et al.*, 2000) and with cereal grains (Rotter *et al.*, 1990; Annison, 1991; Choct and Annison, 1990; Huyghebaert and Schöner, 1999). In addition, the individual structure of NSP such as arabinoxylans can vary between varieties of wheat (Veldman and Vahl, 1994; Austin *et al.*, 1999). Knowledge of the NSP composition of the feedstuffs used in a broiler ration, therefore, is important for determining optimal enzyme supplementation. In addition, developing enzymes which are capable of hydrolyzing the specific polysaccharide linkages which form the NSP would be of

benefit (Chesson, 2000).

2.6.5 Impact of Enzymes on Non-starch Polysaccharide Digestibility

Hesselman and Åman (1986) found that ß-glucanase supplementation of barley diets increased the degradation of ß-glucans in broiler chickens. ß-glucanase supplementation of hull-less barley diets (Jensen *et al.*, 1998) also was shown to increase NSP digestibility in pigs. A different NSP-degrading enzyme was shown by Haberer *et al.* (1998) to increase disappearance of insoluble ß-glucans in mixed diets for pigs. In wheat-fed broilers, Steenfeldt *et al.* (1998b) found excreta digestibility of total NSP to improve with enzyme supplementation. Pettersson and Åman (1989) found that pentosanase supplementation increased digestibility of soluble and insoluble pentosans in wheat-fed broiler chickens. They found NSP digestibility to increase with increasing levels of dietary enzyme. These researchers also noted that, depending upon the relative ability of the enzyme to solubilize and degrade insoluble NSP, a given enzyme actually has the potential to increase digesta viscosity by solubilizing more NSP than it can degrade. This resulted in negative apparent digestibility of soluble pentosans (Pettersson and Åman, 1989). This same phenomenon was observed by Castañón *et al.* (1997) on rye and barley diets supplemented with an enzyme having xylanase and ß-glucanase activity and by Haberer *et al.* (1998) in pigs fed wheat and barley diets with the same supplemented enzyme activities. In most of these studies, NSP content of both the diets and digesta was measured by first removing starch either enzymatically or by washing with ethanol, or both, then hydrolyzing the NSP in concentrated sulphuric acid followed

by derivitization of the sugars to alditol acetates which could then be measured on the gas chromatograph (GC). In only one case were the low molecular weight sugars (mono- and oligosaccharides) recovered from the diet and digesta by extraction with ethanol followed by derivitization and measurement by GC (Steenfeldt *et al.*, 1998b). It would seem relevant, in studies evaluating the effect of enzymes on NSP digestibility, to measure the amount of low molecular weight sugars present in the digesta, since enzymatic hydrolysis of NSP will result in an increased presence of these sugars in the digestive tract. Whether they remain in the tract, are absorbed by the bird, or are fermented by the resident microflora, remains to be determined.

Improvements in NSP digestibility with enzyme supplementation can also be seen in protein ingredients such as canola meal (Slominski and Campbell, 1990), although the specific activity of the enzyme was not mentioned in this study. No effect of three experimental enzymes on NSP digestibility in one variety of lupins was observed while a significant effect was observed with the same enzymes on a second variety of lupins (Kocher *et al.*, 2000). The effects were the same for each enzyme, despite clear differences in their specificities. This demonstrates that the structure of the NSP in the target substrate has a substantial influence on the activity of the enzyme.

The best hydrolysis of canola and soybean meal galactooligosaccharides through *in vitro* enzyme supplementation was obtained with a combination of α-galactosidase and invertase (ß-fructofuranosidase) (Slominski, 1994). *In vivo* experiments with caecectomized laying hens resulted in an average 88% hydrolysis of galacto-oligosaccharides with a similar combination of α-galactosidase (2 g/kg) and invertase (1

g/kg) (Slominski, 1994). The same study revealed a potential problem of dietary minerals inhibiting hydrolysis by α-galactosidase under practical poultry feeding conditions.

Veldman *et al.* (1993) attempted to utilize α-galactosidases to overcome negative digestive effects of high galactooligosaccharide diets in pigs but failed to demonstrate a positive effect. Similarly, Irish *et al.* (1995) were unable to demonstrate performance improvements *in vivo* of supplementing diets with α-galactosidase plus invertase despite having observed significant *in vitro* reduction of soybean meal oligosaccharides. Brenes *et al.* (1993) demonstrated a positive effect of enzyme supplementation on the nutritive value of lupins but these researchers were using a blend of three enzymes with numerous specificities including an "unknown level of α-galactosidase activity", making interpretation of these results difficult. More recently, Hughes *et al.* (2000), using a mixture of two commercially available NSP-degrading enzymes (with pectinase, α-galactosidase, ß-glucanase and endoxylanase activities) in diets with added lupin NSP, demonstrated depolymerization of insoluble NSP which resulted in increased ileal viscosity. No measurement was made on NSP digestibility per se.

The variability in NSP digestibility in response to enzyme supplementation seen in the literature is not unexpected given the substantial variation in the NSP structure of cereals (Veldman and Vahl, 1994; Austin *et al.*, 1999; Huisman *et al.*, 2000) and, therefore, their viscosity generating properties, even within a species (Bedford and Schulze, 1998).

2.6.6 Impact of Enzymes on the Microbial Ecology of the Gastrointestinal Tract

Salih *et al.* (1991) showed that ß-glucanase supplementation of hull-less barley diets tended to decrease total bacterial counts in the jejunum and ileum of broilers. In diets with added soluble arabinoxylans, Choct *et al.* (1995, 1996) demonstrated that dietary xylanase decreased bacterial fermentation in the ileum but increased fermentation in the caeca. These researchers speculated that the enzyme resulted in either reduced viscosity resulting in better access of NSP substrates to the caeca, or in lower molecular weight soluble NSP entering the caeca and being rapidly fermented. Researchers examining the impact of enzyme supplementation on specific groups of bacteria have been few. Hock *et al.* (1997) found that feeding a semi-purified wheat diet supplemented with xylanase resulted in decreased coli-aerogenic bacteria and lactobacilli in the small intestine and no changes in any of the caecal flora evaluated. Vahjen *et al.* (1998) found that xylanase supplementation of wheat-fed broilers resulted in a reduction in luminal *Lactobacillus* numbers in the ileum but an increase in mucosa-associated *Lactobacillus*. Both luminal and mucosa-associated ileal Gram positive cocci were decreased in the small intestine when xylanase was fed. Follow-up work by Dänicke *et al.* (1999) again demonstrated that xylanase supplementation, this time in rye diets, reduced total ileal anaerobes, enterobacteria, Gram positive cocci and enterococci, but only when tallow was the added fat, not soybean oil.

Apajalahti and Bedford (1998), using % G+C analysis (DNA base composition), found that supplementation of a wheat diet with a xylanase decreased clostridia, *Escherichia*, *Salmonella* and *Campylobacter* and increased *Bacteroides* ($P<0.05$),

propionibacteria, eubacteria and bifidobacteria in the caecum of broilers. These researchers also noted that xylanase supplementation decreased the available substrate in the ileum thereby decreasing ileal bacterial populations. In addition, they found that the enzyme resulted in significant increases in total caecal VFAs and, in particular, propionic acid. Choct *et al.* (1999), using normal wheat diets (without added arabinoxylans as were used previously), were also able to demonstrate a significant reduction of ileal fermentation and a significant increase in caecal fermentation with xylanase supplementation. Bedford (1996b) suggests that since caecal fermentation increases while ileal bacterial fermentation decreases with enzyme supplementation, the current xylanase enzymes may increase nutrient utilization by the bird, through reduction of bacterial competition for available substrates in the SI. Support for this hypothesis could be demonstrated by providing evidence of the difference in available NSP substrate at the terminal ileum between unsupplemented and enzyme supplemented wheat diets.

It is clear that, despite the large volume of research in the area of dietary NSP and enzyme supplementation in broiler diets, the mechanisms whereby NSP and enzymes interact with the gastrointestinal tract microflora have not been elucidated.

3.0 INFLUENCE OF DIETARY NON-STARCH POLYSACCHARIDE AND ENDOXYLANASE SUPPLEMENTATION ON ADAPTABILITY OF THE GASTROINTESTINAL TRACT AND THE GASTROINTESTINAL TRACT BACTERIA OF THE BROILER CHICKEN.

3.1 Abstract

Two experiments were conducted to examine the impact of dietary non-starch polysaccharide (NSP), exogenous xylanase supplementation and age on the adaptability of the broiler chicken gastrointestinal tract (GIT) and its resident bacteria. A wheat-based diet with and without added xylanase was compared to a corn-based diet in Experiment 1 and to two additional wheat diets where the xylanase was either added or withdrawn at 28 d in Experiment 2. Birds were sampled at 42 d for GIT lengths and weights and collection of GIT contents for bacterial culturing and volatile fatty acid (VFA) analysis. Experiment 2 involved all of the wheat treatments used in Experiment 1 but birds were sampled at 14, 28 and 42 d for GIT lengths and weights and collection of GIT contents. Bacteria were cultured from 28 and 42 d samples. Birds in both experiments had improved performance when xylanase was used in the wheat diets. Viscosity was lowest for corn diets and was significantly lower in xylanase supplemented diets when compared to unsupplemented diets, except at 42 d. GIT measures were all smaller on corn versus wheat-based diets. Full ileal weights were

higher for unsupplemented wheat diets versus all others while caecal weights were lower on this treatment. In Exp. 1, ileal anaerobes tended to be higher with enzyme supplementation at 42 d than without while caecal anaerobes were higher on unsupplemented wheat diets. In Exp. 2, bacterial data indicated higher levels of ileal anaerobes and some caecal anaerobes on unsupplemented diets at 28 and 42 d. Bacterial fermentation, as measured by VFA content of the digesta, at 28 d showed higher ileal fermentation in diets without enzyme supplementation and the same tendency was noted for caecal fermentation. At 42 d ileal fermentation was higher with enzyme and caecal fermentation was higher without enzyme. These results demonstrate that while certain anaerobic bacteria do increase in the ileum of unsupplemented wheat diets, others appear to respond to the substrates created by enzyme supplementation in both the ileum and the caecum. Age related adaptation also appears to affect the response of the bacteria to enzyme supplementation.

3.2 Introduction

The incorporation of exogenous enzyme products into grain-based diets for broiler chickens is an accepted and proven method for significantly improving both performance and litter quality. What is known of the modes of action of supplementary enzymes has been reviewed periodically in some detail (Campbell and Bedford, 1992; Bedford, 1995; Bedford and Schulze, 1998). While these reviews and the research summarized therein go into detail on the implications of high levels of soluble arabinoxylans and β-glucans increasing digesta viscosity and leading to poorer

digestibility of nutrients (Annison, 1991; Choct and Annison, 1990, 1992a, 1992b; Morgan and Bedford, 1995; Van der Klis *et al.*, 1995) and slowed passage rates (Salih *et al.*, 1991; Dänicke *et al.*, 1997a, 1999), much of the commentary on the involvement of the naturally occurring bacteria of the gastrointestinal tract is educated speculation. Only a very limited number of studies have attempted to evaluate the impact of the GIT flora on either NSP degradation or the improvement seen with enzyme supplementation of high non-starch polysaccharide (NSP) diets (Choct *et al.*, 1995, 1996; Langhout, 1998; Vahjen *et al.*, 1998; Dänicke *et al.*, 1999). The objective of the current study was, therefore, to evaluate age and enzyme related changes in gastrointestinal tract size and in bacterial populations and fermentation patterns in the digestive tract of wheat-fed broiler chickens and to evaluate the relationship of these criteria on enzyme-related improvements in performance.

3.3 Materials and Methods

3.3.1 Bird Management, Diets, Sampling and Gastrointestinal Tract Measures

3.3.1.1 Experiment 1

A total of 1500 male and female, day old broiler chickens (Petersen x Hubbard) were randomly assigned to 3 replicate pens of each sex on each of five dietary treatments. Cleaned and disinfected floor pens bedded with straw accommodated 50 birds each. Birds were cared for using standard management practices of the University of Saskatchewan. Initial room temperature was 35 C and was gradually decreased to 22

C by 35 d. This temperature was maintained to the end of the trial.

Dietary treatments included a corn-based diet, a wheat based diet, and a wheat based diet supplemented with a commercial xylanase (Avizyme 1300: xylanase activity 2700 IU/g, protease activity 800 IU/g; Finnfeeds International, Marlborough, Wiltshire, UK, SN8 1XN). Diets were calculated to be of similar nutrient composition with 3,000 kcal/kg AME, and 22 %, 20 % and 19 % CP, respectively for the starter, grower and finisher diets (Table 3.3.1a,b,c). Additional treatments whereby the enzyme was either added or withdrawn at 28 d were included to evaluate the adaptability of the GIT bacteria. The starter diet was fed from 0-14 d, the grower from 15-28 d and the finisher from 29-42 d. Feed and water were provided *ad libitum*. All diets in Experiment 1 were supplemented with virginiamycin (Pfizer Animal Health Canada, Montreal, QC) at 11 mg/kg diet as an antibiotic growth promotant. Pen weights were taken at each diet change. Feeders were weighed to measure feed consumption. Weight gain and feed conversion efficiency were calculated for each two week period and overall.

At 42 d of age, four birds per replicate were weighed and killed by injection with T-61 euthanasia solution (Embutramide 200mg/mL, Hoechst Roussel Vet Canada Inc., Regina, SK). Intestinal tracts were excised and divided into the jejunum (from duodenum to Meckel's Diverticulum), ileum (from Meckel's Diverticulum to the ileocaecal junction) and paired caeca. Component lengths and full weights were taken, the components gently rolled to extract their contents, then empty weights were taken. The contents were pooled across the 4 birds and subjected to analysis for viscosity and volatile fatty acids. Viscosity was measured on the supernatant of jejunal and ileal

contents using a Brookfield Viscometer (Model DV-III, Brookfield Engineering Laboratories, Inc., Stoughton, MA) following the method described by Bedford and Classen (1993). The remaining samples were frozen at –4 C for subsequent VFA analysis.

Two additional birds per replicate from the corn, wheat and wheat plus enzyme diets were sampled separately for bacteriological evaluation. These birds were also killed in the same manner whereupon the last 10 cm of the ileum and the right caecum were removed, tied off and placed on ice for microbiological processing.

The experimental protocol was approved by the Animal Care Committee, and the procedures were performed in accordance with the requirements of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

3.3.1.2 Experiment 2

A total of 2160 day-old male broiler chickens were randomly allocated to 9 replicate pens of 60 birds for each of four dietary treatments. Dietary treatments included a wheat based diet, and a wheat based diet supplemented with a commercial xylanase (Avizyme 1300) and two additional treatments whereby the enzyme was either added or withdrawn at 28 d, as used in Experiment 1. Diets were the same as the wheat diets used in Experiement 1 (Table 3.3.1a,b,c) except that virginiamycin was not added in an attempt to minimize any impact other than the dietary treatments on the GIT flora. The birds were housed and cared for as in Experiment 1.

Table 3.3.1a. Ingredient and nutrient composition of starter diets (0-14 d) used in experiments 1 and 2

Ingredient	Content (% of Diet)				
	Corn	Wheat	Wheat plus Enzyme (E)	Wheat + E (0-28 d)	Wheat + E (29-42 d)
Wheat	-	61.7	61.5	61.5	61.7
Corn	57.8	-	-	-	-
Soybean meal 48%	35.7	30.7	30.7	30.7	30.7
Canola oil	2.0	3.28	3.33	3.33	3.28
Dicalcium phosphate	1.74	1.62	1.62	1.62	1.62
Limestone	1.34	1.39	1.39	1.39	1.39
Sodium chloride	0.46	0.43	0.43	0.43	0.43
Vit/min premix[1]	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.27	0.19	0.19	0.19	0.19
Enzyme[2]	-	-	0.10	0.10	-
Coccidiostat[3]	0.10	0.10	0.10	0.10	0.10
Calculated composition:					
AME (kcal/kg)	3,000	3,000	3,000	3,000	3,000
CP (%)	22.4	23.0	23.0	23.0	23.0
Ca (%)	1.0	1.0	1.0	1.0	1.0
Av. P (%)	0.45	0.45	0.45	0.45	0.45
Lysine	1.24	1.20	1.20	1.20	1.20
Methionine	0.61	0.53	0.53	0.53	0.53

[1]Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11,000 IU; vitamin D_3, 2,200 IU; vitamin E (dl-α-tocopheryl acetate), 30 IU; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 6 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B_{12}, 20 μg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 150 μg; iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

[2]Avizyme 1300 (Finnfeeds International).

[3]Coxistac (Pfizer Canada Inc.), Note: In Experiment 1 the growth promotant Stafac 22 (Pfizer Canada Inc.) was also included at 0.05 % to provide 11 ppm virginiamycin in the complete diet.

Table 3.3.1b. Ingredient and nutrient composition of grower diets (15-28 d) used in experiments 1 and 2

Ingredient	Content (% of Diet)				
	Corn	Wheat	Wheat plus Enzyme (E)	Wheat + E (0-28 d)	Wheat + E (29-42 d)
Wheat	-	72.2	72.0	72.0	72.2
Corn	63.9	-	-	-	-
Soybean meal 48%	29.8	21.5	21.6	21.6	21.5
Canola oil	2.0	2.08	2.14	2.14	2.08
Dicalcium phosphate	1.78	1.54	1.54	1.54	1.54
Limestone	1.09	1.22	1.22	1.22	1.22
Sodium chloride	0.46	0.42	0.42	0.42	0.42
Vit/min premix[1]	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.18	0.11	0.11	0.11	0.11
L-lysine HCL		0.11	0.11	0.11	0.11
Enzyme[2]	-	-	0.10	0.10	-
Coccidiostat[3]	0.10	0.10	0.10	0.10	0.10
Calculated composition:					
AME (kcal/kg)	3,060	3,000	3,000	3,000	3,000
CP (%)	20.0	20.0	20.0	20.0	20.0
Ca (%)	0.9	0.9	0.9	0.9	0.9
Av. P (%)	0.45	0.43	0.43	0.43	0.43
Lysine	1.08	1.05	1.05	1.05	1.05
Methionine	0.49	0.41	0.41	0.41	0.41

[1]Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11,000 IU; vitamin D_3, 2,200 IU; vitamin E (dl-α-tocopheryl acetate), 30 IU; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 6 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B_{12}, 20 μg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 150 μg; iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

[2]Avizyme 1300 (Finnfeeds International).

[3]Coxistac (Pfizer Canada Inc.), Note: In Experiment 1 the growth promotant Stafac 22 (Pfizer Canada Inc.) was also included at 0.05 % to provide 11 ppm virginiamycin in the complete diet.

Table 3.3.1c. Ingredient and nutrient composition of finisher (29-42 d) diets used in experiments 1 and 2

Ingredient	Content (% of Diet)				
	Corn	Wheat	Wheat plus Enzyme (E)	Wheat + E (0-28 d)	Wheat + E (29-42 d)
Wheat	-	73.9	73.8	73.9	73.8
Corn	63.2	-	-	-	-
Soybean meal 48%	30.0	19.2	19.2	19.2	19.2
Canola oil	2.0	2.17	2.22	2.17	2.22
Dicalcium phosphate	1.78	1.67	1.67	1.67	1.67
Limestone	1.00	0.90	0.90	0.90	0.90
Sodium chloride	0.33	0.30	0.30	0.30	0.30
Vit/min premix[1]	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.07	0.08	0.08	0.08	0.08
Enzyme[2]	-	-	0.10	-	0.10
Coccidiostat[3]	0.10	0.10	0.10	0.10	0.10
Celite[4]	1.0	1.0	1.0	1.0	1.0
Calculated composition:					
AME (kcal/kg)	3,037	3,000	3,000	3,000	3,000
CP (%)	20.0	19.0	19.0	19.0	19.0
Ca (%)	0.8	0.8	0.8	0.8	0.8
Av. P (%)	0.45	0.45	0.45	0.45	0.45
Lysine	1.09	0.90	0.90	0.90	0.90
Methionine	0.38	0.38	0.38	0.38	0.38

[1]Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11,000 IU; vitamin D_3, 2,200 IU; vitamin E (dl-α-tocopheryl acetate), 30 IU; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 6 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B_{12}, 20 μg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 150 μg; iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

[2]Avizyme 1300 (Finnfeeds International).

[3]Coxistac (Pfizer Canada Inc.), Note: In Experiment 1 the growth promotant Stafac 22 (Pfizer Canada Inc.) was also included at 0.05 % to provide 11 ppm virginiamycin in the complete diet.

[4]As an Acid Insoluble Ash marker, in Experiment 1 only (Celite Corp., Lompac, CA 93436), For Experiment 2 compositions, add 1 % of major grain back to diet.

Birds were killed, weighed and sampled in the same manner as Experiment 1 at 14, 28 and 42 d of age for gastrointestinal tract measures and content collection. Intestinal segments for bacterial culturing were also collected in the same manner as Experiment 1 except that only one bird from each of 8 replicates was sampled for bacterial culturing at 28 and 42 d of age.

The experimental protocol was approved by the Animal Care Committee, and the procedures were performed in accordance with the requirements of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

3.3.2 Bacteriology

Samples of ileal and caecal contents were weighed into sterile, conical dilution tubes with peptone water and cysteine hydrochloride in a laminar flow hood. They were serially diluted with a solution of peptone water and cysteine hydrochloride and cultured for aerobic enterobacteria by plating on BBL MacConkey Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubating at 37 C for 24 h. They were also cultured for microaerophilic *Lactobacillus* spp. by plating on BBL LBS Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubating the plates in anaerobic jars at 37 C for 48 h and for anaerobic *Clostridium* spp. and *Bifidobacterium* spp. by plating on BBL Clostrisel (Becton Dickinson Microbiology Systems, Cockeysville, MD) and Bereen's Agar and incubating in an anaerobic hood at 37 C for 48 h. The Bereen's agar was made from BBL Columbia Agar Base (Becton Dickinson Microbiology Systems, Cockeysville, MD). Plating was done using an

Autoplate 4000 (Spiral Biotech, Bethesda, MD) spiral diluter. Colonies on incubated plates were counted either by hand using the Spiral Biotech sector counting method, or using a Colony Image Analyser (Spiral Biotech, Bethesda, MD) calibrated for each type of bacterial plate.

For Experiment 2, anaerobic caecal *Bacteroides* were cultured instead of aerobic caecal enterobacteria. These were plated on Bacteroides Bile Esculin Agar and incubated for 48 h at 37 C in an anaerobic hood.

3.3.3 Volatile Fatty Acid Analysis

Sub-samples of jejunal (0.5 g) (Experiment 1 only), ileal (0.5 g) and caecal (0.2 g) contents were taken, vortexed with 1 ml of prepared internal standard (crotonic acid in Experiment 1; isocaproic acid in Experiment 2) solution, and centrifuged at 15,900 x g. Volatile fatty acids were measured on the supernatant using a gas chromatograph (GC) (Varian Star 3400Cx with a Varian 8200Cx autosampler, Varian, Walnut Creek, CA). The column was a glass capillary column packed with carbowax fused silica (Stabilwax-DAS, RESTEK Corporation, Bellefonte, PA). The injector temperature on the GC was 220 C, the initial column temperature was 140 C. This temperature was held for 5 minutes, then was gradually increased to a final column temperature of 220 C. The flame ionization detector temperature of the GC was 230 C. Results were expressed as mmol/L and were converted to μmol/g of wet digesta content. The method used was a modification of Corrier *et al.* (1990a) with the modifications being adaptations for use with a capillary column and different internal standards, as outlined above.

3.3.4 Statistical Analysis

Experiment 1 was analysed as a two-way analysis of variance using the general linear models procedure of SAS (SAS Institute, 1989) to determine significant effects of diet and sex of the bird. Significant mean differences ($P<0.05$) were determined using Duncan's multiple range test (Steel and Torrie, 1980). The bacterial data was log transformed prior to statistical analysis. In Experiment 2, the analysis was a two-way analysis of variance looking at age, diet and interactions. Predetermined orthogonal contrasts were also used on the bacterial data to compare enzyme supplemented and unsupplemented wheat diets.

3.4 Results

3.4.1 Performance

Since no treatment by sex interactions were observed, sexes were pooled and only treatment main effects are presented for Experiment 1. In Experiment 1, the corn treatment resulted in better bird performance than any wheat diet (Figure 3.4.1a). Enzyme supplementation of wheat diets resulted in improved overall feed efficiency (Table 3.4.1) over unsupplemented diets. The first experiment also demonstrated performance improvements, equivalent to full (42 d) enzyme supplementation, with enzyme addition as late as 28 d of age. In Experiment 2, the performance of birds with enzyme addition at 28 d was not significantly greater than the unsupplemented birds, whereas full (42 d) enzyme supplementation did result in superior performance (Figure 3.4.1b; Table 3.4.1). Performance of birds where enzyme was withdrawn at 28 d, in

Table 3.4.1 Effect of diet on overall performance of broilers in Experiments 1 & 2

Variables		Performance Parameters at 42 d		
		Avg Gain (kg)	Avg Feed Cons. (kg)	Gain to Feed[1]
Diet Expt 1	Corn	2.393[a]	4.907[c]	0.560[a]
	Wheat	2.239[b]	5.374[a]	0.492[c]
	Wheat plus Enzyme	2.241[b]	5.122[bc]	0.510[b]
	Wheat plus Enzyme 0-28 d	2.202[c]	5.254[ab]	0.501[c]
	Wheat plus Enzyme 29-42 d	2.272[b]	5.205[ab]	0.513[b]
Statistics				
Diet		***	**	***
SEM		0.036	0.094	0.005
Diet Expt 2	Wheat	2.527	6.419	0.495[b]
	Wheat plus Enzyme	2.569	6.208	0.526[a]
	Wheat plus Enzyme 0-28 d	2.528	6.244	0.502[ab]
	Wheat plus Enzyme 29-42 d	2.488	6.557	0.492[b]
Statistics				
Diet		NS	NS	*
SEM		0.013	0.112	0.005

[1]Mortality corrected

[a-c] Means within columns and experiment with no common superscript differ significantly (*=$P<0.05$;**=$P<0.01$;***=$P<0.001$; NS = not significant).

SEM = pooled standard error of the mean

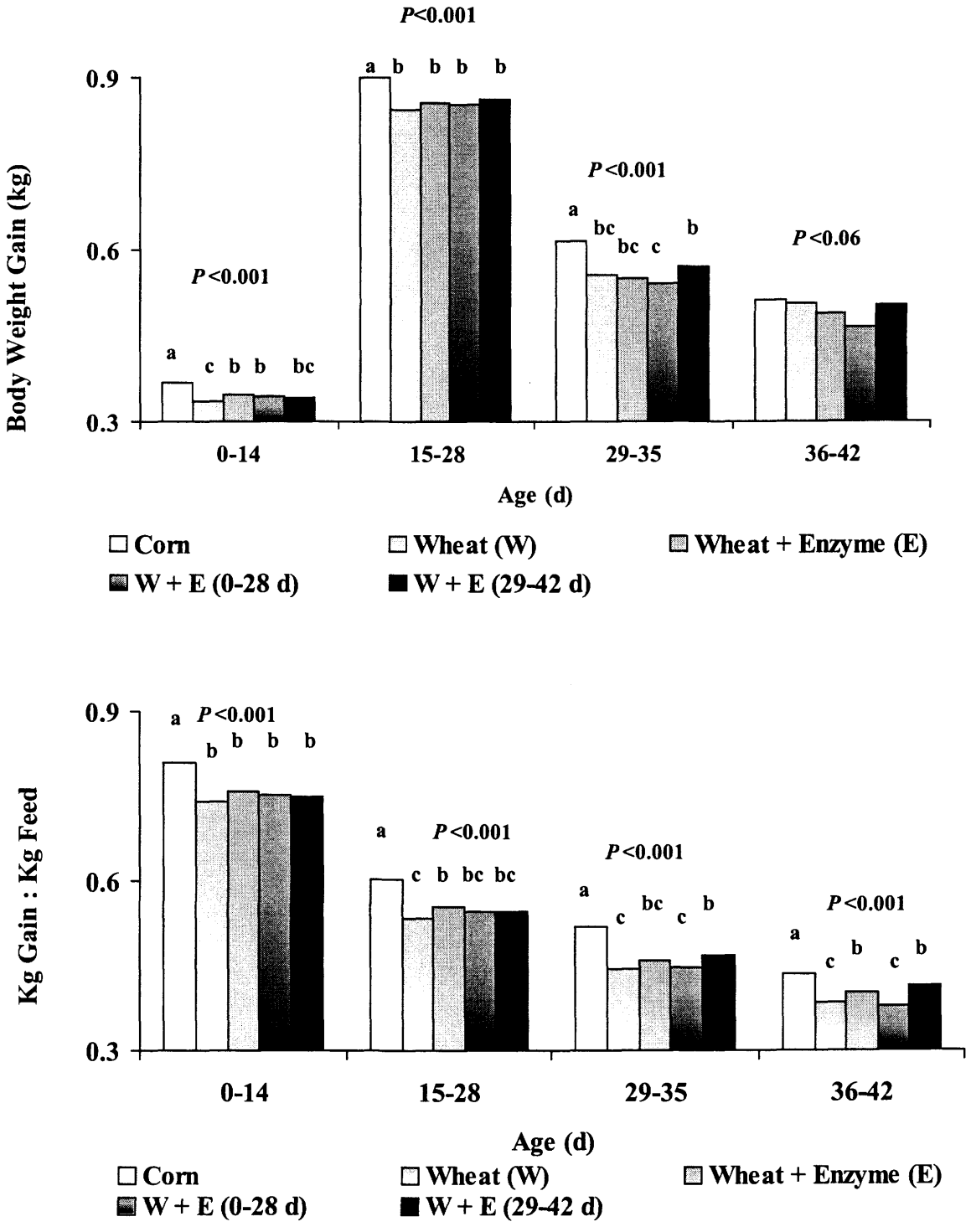

Figure 3.4.1a. Effect of dietary treatment on the growth rate and feed efficiency of broiler chickens in experiment 1

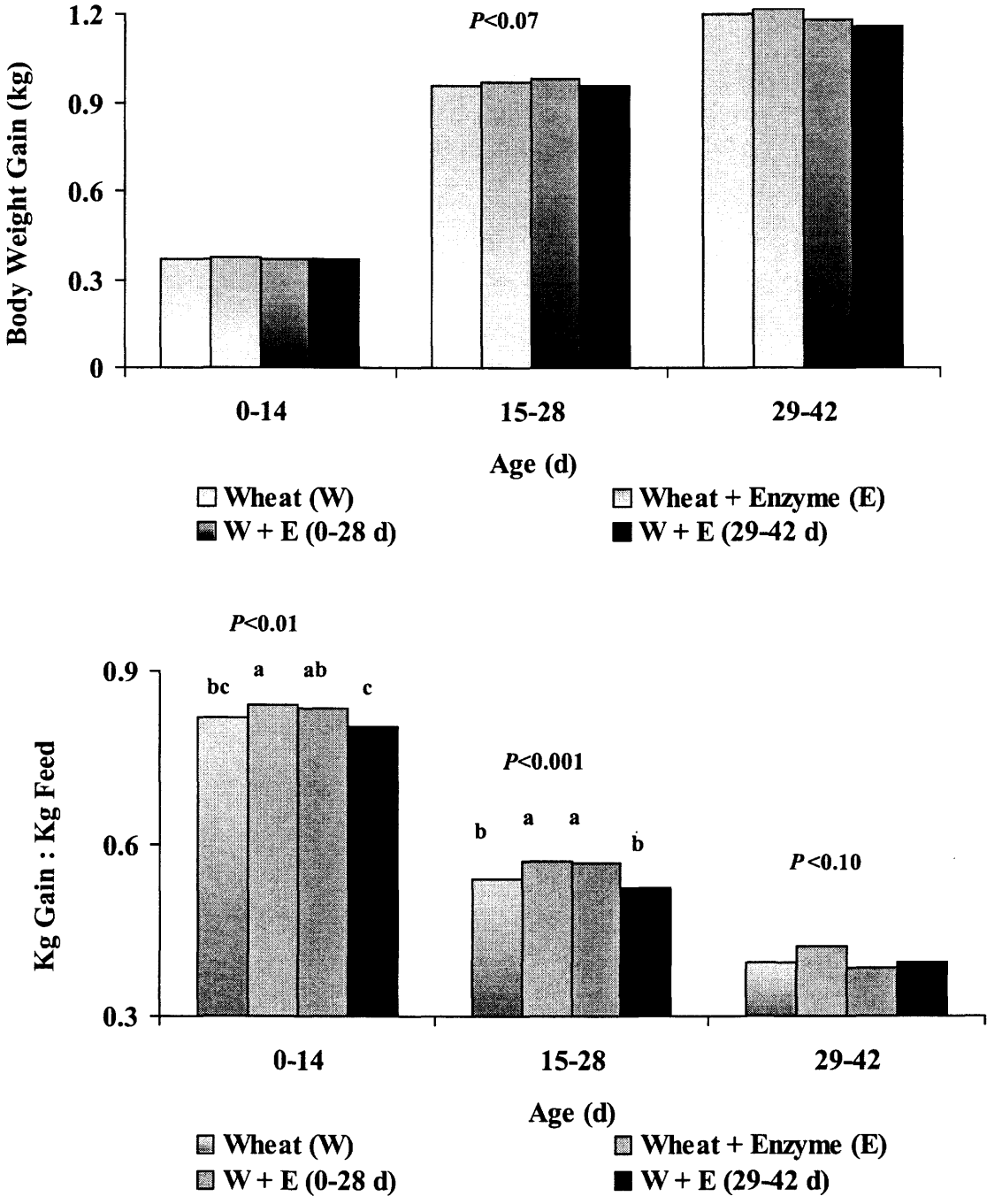

Figure 3.4.1b. Effect of dietary treatment on the growth rate and feed efficiency of broiler chickens in Experiment 2

both experiments, was equivalent to unsupplemented birds. There were no treatment effects on bird mortality.

3.4.2 Viscosity

Small intestinal (SI) viscosity was measured at 42 d only in Experiment 1 and at 14, 28 and 42 d in Experiment 2. The corn diet in Experiment 1 resulted in the lowest jejunal and ileal viscosity (Table 3.4.2). In Experiment 2, enzyme use decreased viscosity significantly in both the jejunum and the ileum (Table 3.4.3a). There was also a significant age effect showing a decrease in jejunal viscosity between 28 and 42 d while the highest ileal viscosity occurred at 28 d, followed by a drop. Diet by age interactions were significant and are shown in Table 3.4.3b. Enzyme use decreased viscosity significantly at 14 and 28 d (Table 3.4.3b) and numerically at 42 d (Tables 3.4.2 and 3.4.3b). Treatments where enzyme was withdrawn at 28 d yielded numerically similar or higher SI viscosities by 42 d than birds fed unsupplemented diets throughout the trial. Those where enzyme was added at 28 d yielded SI viscosities similar to birds supplemented throughout the experiment. The effects of the change in enzyme supplementation were evident within a matter of hours after the diets were switched as sampling of the birds was carried out over the course of the afternoon. The diets were changed in the morning after bird weights and feeds consumption had been measured. The timing of these measures, while not ideal, was necessary to ensure that GIT fill was maintained and that feed consumption measures were accurate.

From the viscosity data (Table 3.4.3b) in Experiment 2, jejunal and ileal viscosity

Table 3.4.2 Dietary treatment effects on viscosity and size of the gastrointestinal tract, relative to body weight, in 42 d old broiler chickens in Experiment 1

Variables	Gastrointestinal Tract Size[1] and Digesta Viscosity[2]										
	Jejunum				Ileum				Caecal		
	Viscosity	Length	Wt Full	Wt Empty	Viscosity	Length	Wt Full	Wt Empty	Length	Wt Full	Wt Empty
Corn	1.97^{c}	3.14^{b}	2.17^{b}	1.09^{b}	2.22^{b}	3.16^{b}	1.67^{b}	0.97^{b}	1.50^{b}	0.59^{c}	0.38^{b}
Wheat (W)	3.11^{ab}	3.51^{a}	2.60^{a}	1.28^{a}	5.14^{a}	3.56^{a}	2.11^{a}	1.15^{a}	1.66^{a}	0.63^{bc}	0.43^{ab}
Wheat plus Enzyme (E)	2.67^{b}	3.45^{a}	2.50^{a}	1.25^{a}	4.24^{a}	3.50^{a}	2.04^{a}	1.16^{a}	1.72^{a}	0.71^{ab}	0.47^{a}
W + E 0-28 d	3.57^{a}	3.62^{a}	2.35^{ab}	1.20^{a}	4.27^{a}	3.62^{a}	1.88^{ab}	1.09^{a}	1.71^{a}	0.74^{a}	0.46^{a}
W + E 29-42 d	3.11^{ab}	3.45^{a}	2.44^{a}	1.20^{a}	3.56^{ab}	3.60^{a}	1.94^{a}	1.09^{a}	1.68^{a}	0.71^{ab}	0.44^{ab}
Statistics											
Diet	**	***	**	**	**	***	**	**	***	*	*
SEM	0.136	0.041	0.040	0.039	0.282	0.043	0.039	0.017	0.022	0.016	0.009

[1](value/body weight) X 100.
[2]cps.
[a-c]Means within a column with differing superscripts are significantly different (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$).
SEM = pooled standard error of the mean.

Table 3.4.3a Main effects of diet and age on jejunal and ileal digesta viscosity in wheat fed broiler chickens in Experiment 2

Variables		Digesta Viscosity (cps)	
		Jejunal	Ileal
Diet	Wheat	3.90[a]	6..26[a]
	Wheat plus Enzyme	2.82[c]	4.02[c]
	Wheat plus Enzyme (0-28 d)	3.21[b]	4.49[bc]
	Wheat plus Enzyme (29-42 d)	3.31[b]	4.82[b]
Age (d)	14	3.43[a]	4.70[b]
	28	3.47[a]	5.51[a]
	42	3.03[b]	4.49[b]
Statistics			
	Diet	***	***
	Age	*	***
	Diet X Age	0.06	***
	SEM	0.082	0.157

[a,b,c] Means, within a column and main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; *** = $P \leq 0.001$)
SEM = pooled standard error of the mean.

Table 3.4.3b Effect of xylanase supplementation and age on jejunal and ileal digesta viscosity of broiler chickens in Experiment 2

Gut Segment	Age (d)	Treatments				*P*	SEM
		Wheat	Wheat plus Enzyme	Wheat plus Enzyme (0-28 d)	Wheat plus Enzyme (29-42 d)		
Jejunum	14	4.25[a]	2.78[b]	2.93[b]	3.77[a]	**	0.28
	28	4.21[a]	2.93[b]	3.46[ab]	3.28[a]	*	0.42
	42	3.23	2.75	3.25	2.89	NS	0.16
Ileum	14	6.06[a]	3.76[b]	3.63[b]	5.36[a]	***	0.42
	28	8.10[a]	4.07[b]	4.77[b]	5.08[b]	***	0.43
	42	4.60	4.24	5.07	4.02	NS	0.31

[a,b] Means within rows with no common superscript differ significantly (* = $P \leq 0.05$; ** = $P<0.01$; *** = $P<0.001$; NS = not significant).
SEM = pooled standard error of the mean.

from birds fed the unsupplemented diets are lowest by 42 d of age. This is not seen in the enzyme supplemented birds. Ileal viscosity is highest for unsupplemented, wheat-fed birds at both 14 and 28 d.

3.4.3 Gastrointestinal Tract Measures

The gastrointestinal tracts of the birds in Experiment 1 were significantly shorter and lighter by 42 d on the corn-based diet (Table 3.4.2). Full weights, relative to body weight, of all segments were lower on the corn diet. No significant differences were noted between wheat-based treatments except that full caecal weights were significantly lower for the unsupplemented wheat diet than for the diet where enzyme was withdrawn at 28 d and numerically lower than the other wheat diets.

Numeric trends of note for the wheat-based diets in Experiment 1 include a tendency for full jejunal and ileal weights to be higher without enzyme and full caecal weights to be higher with enzyme at 42 d. This trend is repeated in Experiment 2 and is actually a significant treatment effect for full ileal weight (Table 3.4.4). As a result, ileal content weights are signficantly highest for birds on the unsupplemented wheat diet and caecal contents were lowest for this treatment. Overall, jejunal and ileal lengths were numerically highest ($P > 0.05$) for the unsupplemented wheat diet. No differences in caecal length were noted (Table 3.4.4). All relative segment lengths analyses show the diets with changes to enzyme supplementation having lengths similar or intermediate to the unchanged treatments.

Relative lengths and weights of all segments decreased with age in Experiment 2

Table 3.4.4 Main effects of xylanase supplementation and age on gastrointestinal tract measures, relative to body weight, in broiler chickens in Experiment 2

Variables		Selected Gastrointestinal Tract Measures[1]											
		Jejunum				Ileum				Caeca			
		Length	Wt Full	Wt Empty	Contents	Length	Wt Full	Wt Empty	Contents	Length	Wt Full	Wt Empty	Contents
Diet	Wheat (W)	6.98	3.33	1.82	1.51	6.62	2.68^{a}	1.32	1.36^{a}	2.79	0.69	0.48	0.21^{b}
	Wheat + Enzyme (E)	6.67	3.19	1.79	1.40	6.32	2.46^{b}	1.29	1.17^{b}	2.74	0.74	0.48	0.26^{a}
	W + E 0-28 d	6.89	3.25	1.80	1.44	6.59	2.50^{b}	1.29	1.21^{b}	2.84	0.72	0.49	0.24^{ab}
	W + E 29-42 d	6.89	3.33	1.79	1.54	6.49	2.50^{b}	1.27	1.22^{b}	2.79	0.72	0.48	0.24^{ab}
Age (d)	14	12.34^{a}	4.23^{a}	2.41^{a}	1.83^{a}	11.27^{a}	3.19^{a}	1.58^{a}	1.61^{a}	4.65^{a}	0.79^{a}	0.58^{a}	0.21^{b}
	28	5.19^{b}	3.08^{b}	1.60^{b}	1.49^{b}	5.00^{b}	2.41^{b}	1.24^{b}	1.16^{b}	2.23^{b}	0.70^{b}	0.45^{b}	0.25^{a}
	42	3.05^{c}	2.50^{c}	1.39^{c}	1.11^{c}	3.25^{c}	2.01^{c}	1.05^{c}	0.95^{c}	1.49^{c}	0.67^{c}	0.41^{c}	0.26^{a}
Statistics													
	Diet	0.06	NS	NS	0.10	0.06	*	NS	**	NS	NS	NS	*
	Age	***	***	***	***	***	***	***	***	***	***	***	**
	Diet X Age	NS	NS	NS	0.10	NS	NS	NS	NS	NS	NS	NS	NS
	SEM	0.196	0.043	0.023	0.026	0.171	0.035	0.014	0.024	0.068	0.009	0.006	0.006

[1](value/body weight) X 100.

[a-c]Means within a column, within a Diet or Age, with differing superscripts are significantly different (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; NS = not significant); SEM = pooled standard error of the mean.

(Table 3.4.4).

3.4.4 Bacteriology

There were no significant treatment effects on ileal bacteria in the first experiment (analyzed only at 42 d). Enzyme supplemented, wheat-fed birds showed a numerically higher number of ileal microaerophilic *Lactobacillus* spp. (P=0.09), *Bifidobacterium* spp. (P=0.12) and *Clostridium* spp. versus corn-fed and unsupplemented wheat-fed birds (Table 3.4.5). Ileal enterobacteria were not different between treatments. In the caeca, *Lactobacillus* spp. numbers were significantly lower in the corn-fed versus the unsupplemented wheat-fed birds. *Clostridium* spp. were numerically higher in the caeca of unsupplemented wheat-fed birds than of either other treatment.

The bacterial data from Experiment 2 indicated no significant treatment effects (Table 3.4.6). Pre-determined orthogonal contrasts between log transformed bacterial numbers from unsupplemented and enzyme-supplemented wheat-fed birds did reveal higher numbers of ileal enterobacteria as well as numerically higher caecal *Bacteroides* spp. (P=0.08) and *Clostridium* spp. (P=0.07) at 28 d in unsupplemented wheat-fed birds (Table 3.4.7). Caecal *Lactobacillus* spp. also tended (P=0.15) to be higher in these diets at 28 d.

Only ileal enterobacteria increased from 28 to 42 d of age (Table 3.4.6). None of the other bacteria examined were affected by age in Experiment 2. The significant age by diet interaction is shown in Figure 3.4.2. Ileal enterobacteria increase on each treatment between 28 and 42 d except in the case of the late enzyme addition where no change in

Table 3.4.5 Main effects of diet on numbers of selected groups of bacteria in 42 d old broiler chickens in Experiment 1

Variables	Selected Bacterial Groups (Log $_{10}$ CFU/g wet Digesta)							
	Ileum				Caeca			
	Enterobacteria	*Lactobacillus*	Clostridia	Bifidobacteria	Enterobacteria	*Lactobacillus*	Clostridia	Bifidobacteria
Diet								
Corn	4.7	7.97[b]	7.7	7.8	7.4	8.5[b]	8.3	7.8
Wheat	4.9	8.04[ab]	7.6	7.6	7.2	9.2[a]	8.8	8.2
Wheat plus Enzyme	4.8	8.56[a]	8.1	8.3	7.0	8.9[ab]	8.4	8.1
Statistics								
P value	NS	0.09	NS	0.12	NS	*	NS	NS
SEM	0.221	0.119	0.154	0.189	0.122	0.162	0.202	0.125

[a,b] Means within a column with differing superscripts are significantly different (* = $P<0.05$; NS = not significant).
SEM = pooled standard error of the mean.

Table 3.4.6 Main effects of xylanase supplementation and age on numbers of selected groups of bacteria in broiler chickens in Experiment 2

Variables		Selected Bacterial Groups (Log $_{10}$ CFU/g wet Digesta)							
		Ileum				Caeca			
		Enterobacteria	*Lactobacillus*	Clostridia	Bifidobacteria	*Bacteroides*	*Lactobacillus*	Clostridia	Bifidobacteria
Diet	Wheat	4.58	7.10	6.54	6.07	6.33[a]	9.13	8.94	7.15
	Wheat plus Enzyme	4.51	6.90	6.44	5.68	5.65[b]	8.54	8.31	6.53
	Wheat plus Enzyme 0 - 28 d	4.55	7.34	6.87	6.07	6.09[ab]	8.59	8.42	6.62
	Wheat plus Enzyme 29 – 42 d	4.73	7.34	6.94	6.03	6.33[a]	8.80	8.71	6.81
Age (d)	28	4.31[b]	7.26	6.76	6.02	6.12	8.86	8.83	6.84
	42	4.78[a]	7.07	6.63	5.89	6.09	8.67	8.36	6.72
Statistics									
Diet		NS	NS	NS	NS	0.07	NS	NS	NS
Age		**	NS	NS	NS	NS	NS	NS	NS
Diet X Age		*	NS	NS	NS	NS	NS	NS	NS
SEM		0.105	0.118	0.153	0.130	0.105	0.131	0.147	0.149

[a,b] Means within a column, within Diet or Age, with differing superscripts are significantly different (* = $P<0.05$; ** = $P< 0.01$; NS = not significant).
SEM = pooled standard error of the mean.

Table 3.4.7 Orthogonal contrasts between numbers of selected bacterial groups from the ileum and caecum of broiler chickens fed wheat diets with or without xylanase supplementation in Experiment 2

Variables		Selected Bacterial Groups (Log $_{10}$ CFU/g wet Digesta)							
		Ileum				Caeca			
		Enterobacteria	*Lactobacillus*	Clostridia	Bifidobacteria	*Bacteroides*	*Lactobacillus*	Clostridia	Bifidobacteria
Age	Diets								
	No E[1]	4.61[a]	7.20	6.61	5.85	6.40	9.13	9.19	7.00
28 d	E[2]	3.89[b]	7.33	6.90	6.19	5.82	8.60	8.47	6.67
	Contrast	*	NS	NS	NS	0.08	NS	0.07	NS
	No E[3]	4.87	7.02	6.55	5.99	6.10	8.81	8.62	6.76
42 d	E[4]	4.68	7.12	6.71	5.80	6.07	8.53	8.10	6.67
	Contrast	NS	NS	NS	NS	NS	NS	NS	NS

[1]No E at 28 d is the combined means of the Wheat diet and the Wheat + Enzyme (29-42 d) diet.
[2]E at 28 d is the combined means of the Wheat + Enzyme diet and the Wheat + Enyzme (0-28 d) diet.
[3]No E at 42 d is the combined means of the Wheat diet and the Wheat + Enzyme (0-28 d) diet.
[4]E at 42 d is the combined means of the Wheat + Enzyme diet and the Wheat + Enzyme (29-42 d) diet.
[a, b]Means with differing superscripts within a column and within age differ significantly (*= $P<0.05$; NS = non-significant).

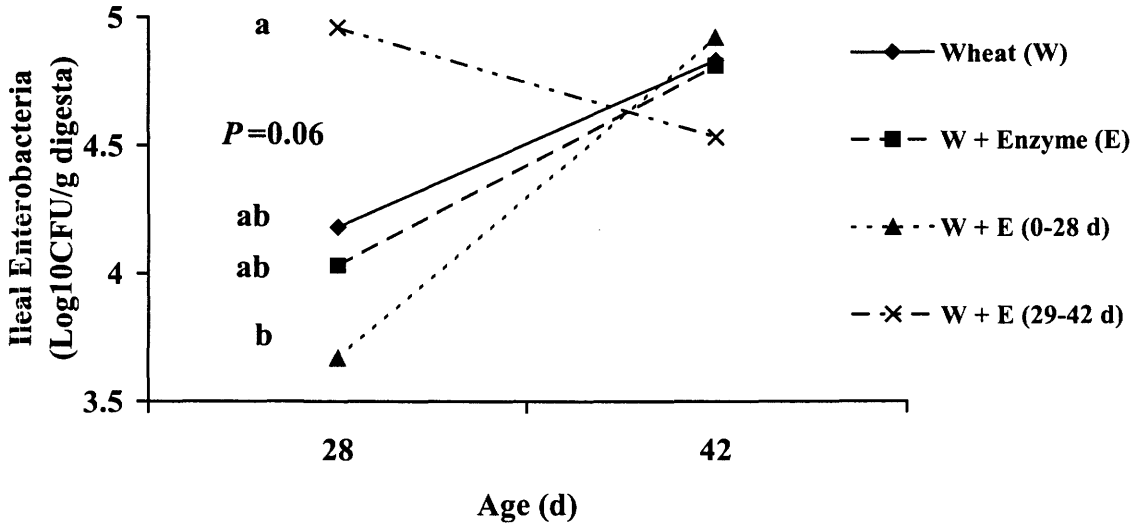

Figure 3.4.2. Effect of xylanase supplementation and age on ileal enterobacteria numbers in the wheat-fed broiler chicken in Experiment 2

number is observed.

3.4.5 Volatile Fatty Acids

In Experiment 1, VFA analysis showed numerically lower jejunal and significantly lower ileal acetic acid levels (Table 3.4.8) on corn versus wheat diets. There were no significant dietary effects on caecal acetic, butyric or total VFA levels. Caecal propionic acid, however, was significantly higher in birds fed corn and the enzyme withdrawal birds than in enzyme supplemented or late enzyme addition birds. Unsupplemented wheat diets yielded numerically higher caecal acetic, propionic, and valeric acid than enzyme supplemented wheat diets. The enzyme withdrawal diet yielded numerically the highest caecal acetic and butyric acid levels as well as significantly higher caecal propionic and valeric acid levels than the enzyme supplemented and the late enzyme addition wheat diets. Of note is the observation that acetic acid levels were much higher in the jejunum than in the ileum in this experiment. Caecal levels were substantially higher than either small intestine segment.

In Experiment 2, a number of diet by age interactions were observed (Table 3.4.9). Ileal acetic acid levels were lower, overall, than those observed in Experiment 1. At 14 d ileal acetic acid (Figure 3.4.3) appears to be higher for the two treatments where the enzyme supplementation changed at 28 d, lower for the unsupplemented wheat diet and lowest for the wheat diet with enzyme supplementation. Overall, ileal acetic acid increased to 28 d, then decreased on all

Table 3.4.8 Effect of dietary NSP and xylanase supplementation on volatile fatty acid levels in the jejunal, ileal and caecal digesta of 42 d broiler chickens in Experiment 1

Variables		Volatile Fatty Acid Levels (µmol/g wet digesta)						
		Jejunal Acetic	Ileal Acetic	Caecal Acetic	Caecal Propionic	Caecal Butyric	Caecal Valeric	Total Caecal VFA
Diet								
	Corn	11.83	4.27[b]	84.22	6.08[a]	30.89	1.98	123.16
	Wheat	17.33	8.33[a]	98.31	4.91[ab]	30.50	1.42	135.14
	Wheat plus Enzyme	20.95	8.48[a]	92.04	3.45[b]	30.68	1.14	127.31
	Wheat plus Enzyme 0 – 28 d	16.68	6.94[ab]	104.89	5.88[a]	42.27	1.98	155.02
	Wheat plus Enzyme 0 – 42 d	17.20	9.71[a]	86.86	3.64[b]	31.34	1.30	123.14
Statistics								
P value		NS	*	NS	*	NS	0.06	NS
SEM		0.749	0.521	3.064	0.251	1.354	0.089	5.082

[a,b] Means within a column with differing superscripts are significantly different (* = $P<0.05$; NS = not significant).
SEM = pooled standard error of the mean.

Table 3.4.9 Effect of xylanase supplementation and age on volatile fatty acid levels in the ileal and caecal digesta of broiler chickens in Experiment 2

Variables		Volatile Fatty Acid Levels (μmol/g wet digesta)											
		Ileal					Caecal						
		Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Total VFA	Acetic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Isovaleric Acid	Valeric Acid	Total VFA
Diet	Wheat	6.13	0.03^{b}	0.01	0.02^{b}	6.53	31.86^{b}	1.98^{a}	0.70	14.74	0.56	0.78	50.61^{b}
	W + Enzyme (E)	3.66	3.12^{a}	0.12	0.18^{a}	7.36	21.76^{b}	1.19^{b}	0.96	12.59	0.52	0.72	37.73^{b}
	W + E 0 – 28 d	5.95	0.43^{b}	0.02	0.03^{b}	6.63	43.30^{a}	2.12^{a}	0.68	16.59	0.48	0.78	63.95^{a}
	W + E 29 – 42 d	6.37	0.28^{b}	0.02	0.02^{b}	6.90	27.69^{b}	1.72^{ab}	0.89	13.73	0.52	0.63	45.16^{b}
Age (d)	14	5.08^{b}	0.02^{b}	0.01^{b}	0.05	5.35^{b}	22.59^{b}	1.84^{a}	0.33^{c}	10.45^{b}	0.36^{b}	0.28^{c}	35.85^{b}
	28	6.83^{a}	0.38^{b}	0.01^{b}	0.03	7.49^{a}	33.04^{a}	1.28^{b}	1.33^{a}	15.42^{a}	0.56^{a}	0.80^{b}	52.43^{a}
	42	4.42^{b}	3.28^{a}	0.13^{a}	0.12	8.36^{a}	37.83^{a}	2.14^{a}	0.75^{b}	17.36^{a}	0.64^{a}	1.10^{a}	59.80^{a}
Statistics													
	Diet	0.07	***	NS	*	NS	***	**	NS	NS	NS	NS	***
	Age	**	***	0.09	NS	*	**	**	***	***	***	***	***
	Diet X Age	**	***	NS	NS	***	***	*	***	NS	NS	NS	**
	SEM	0.391	0.305	0.022	0.023	0.465	2.213	0.110	0.077	0.689	0.025	0.046	2.769

[a-c] Means within a column, within a Diet or Age, with differing superscripts are significantly different (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$; NS = not significant). SEM = pooled standard error of the mean.

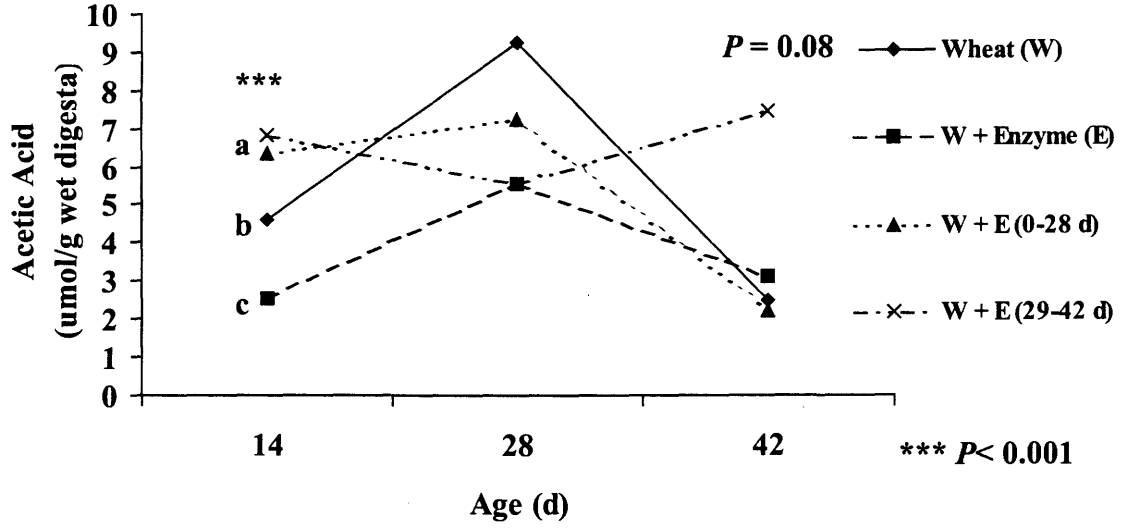

Figure 3.4.3. Effect of xylanase supplementation and age on ileal acetic acid in the wheat-fed broiler chicken in Experiment 2

diets, except for the late enzyme addition diet, where it remained more or less constant.

Ileal propionic acid (Figure 3.4.4) increases with age on all diets with the enzyme supplemented wheat diet resulting in much higher propionic acid levels than all other diets at both 28 and 42 d of age.

The interaction between diet and age seen with total ileal VFA levels (Figure 3.4.5) reflects the patterns observed with ileal acetic and propionic acids, as these make up the largest proportion of total ileal VFA. The 14 d differences are the same as those observed with acetic acid. At 28 d, some VFA are increasing while others are decreasing, resulting in similar total VFA for all treatments. By 42 d, treatments with enzyme supplementation have higher total ileal VFA than those without enzyme.

Ileal butyric and valeric acid levels were low and no interactions were observed (Table 3.4.9). Ileal butyric acid was affected by age and was highest at 42 d, while ileal valeric acid was affected by diet and was highest for the unsupplemented wheat diet overall.

Significant diet by age interactions were also seen for caecal acetic, propionic, iso-butyric and total caecal VFAs (Table 3.4.9). Caecal acetic acid levels (Figure 3.4.6) were again much lower than those measured in Experiment 1. At 14 d there were no treatment differences. At 28 d, the enzyme withdrawal diet (Wheat + E (0-28 d)) had significantly higher levels than the other diets. By 42 d, the two unsupplemented diets both had significantly higher caecal acetic acid levels than the enzyme supplemented wheat diet, with the late enzyme addition diet (Wheat + E (29

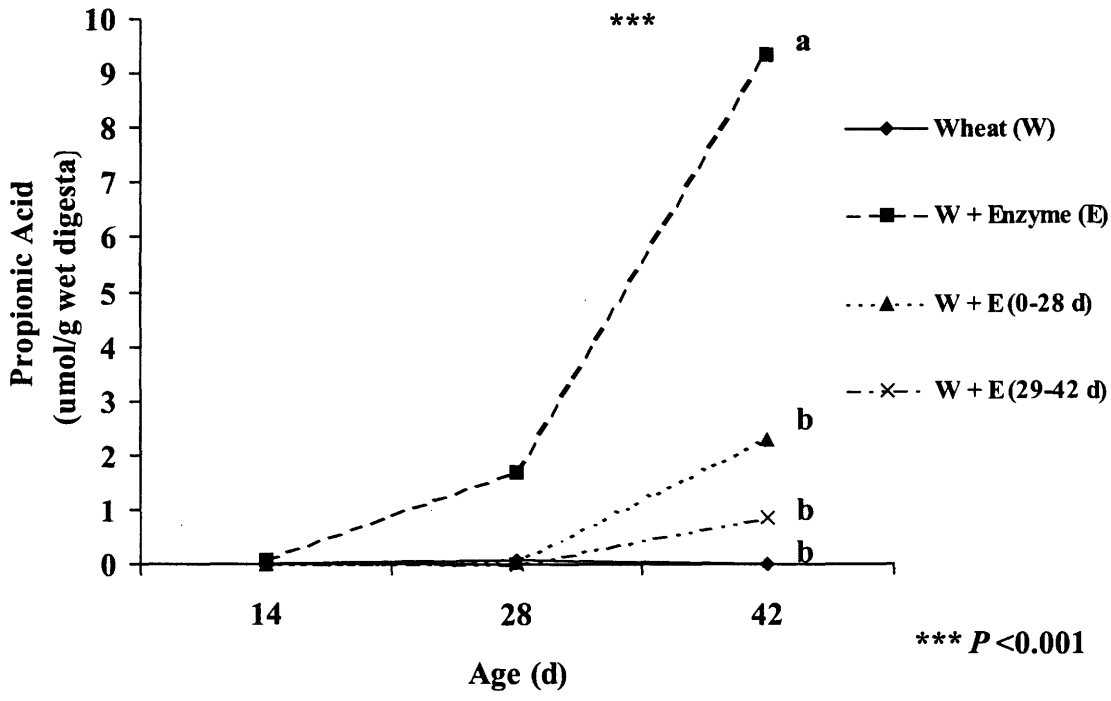

Figure 3.4.4. Effect of xylanase supplementation and age on ileal propionic acid in the wheat-fed broiler chicken in Experiment 2

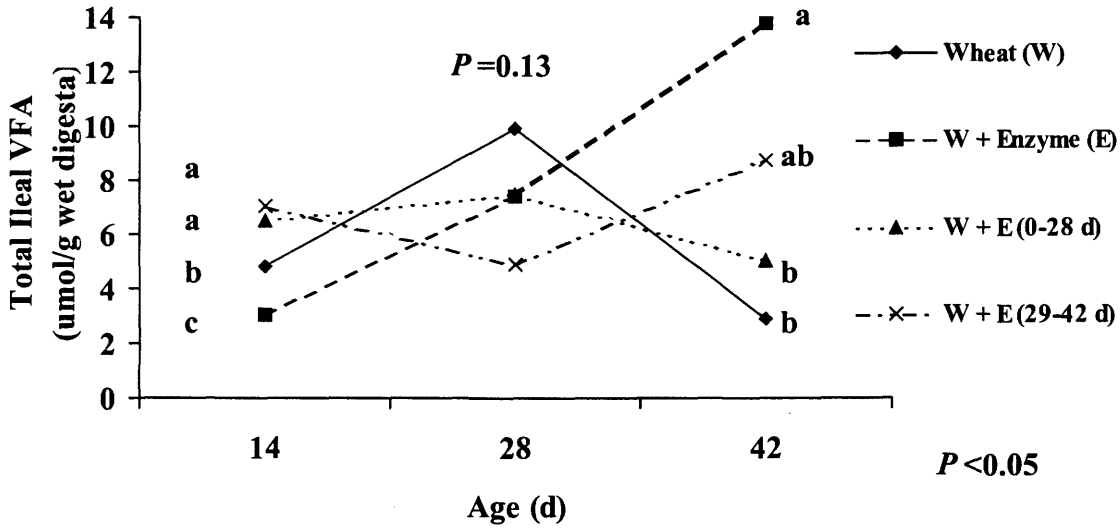

Figure 3.4.5. Effect of xylanase supplementation and age on total ileal volatile fatty acids in the wheat-fed broiler chicken in Experiment 2

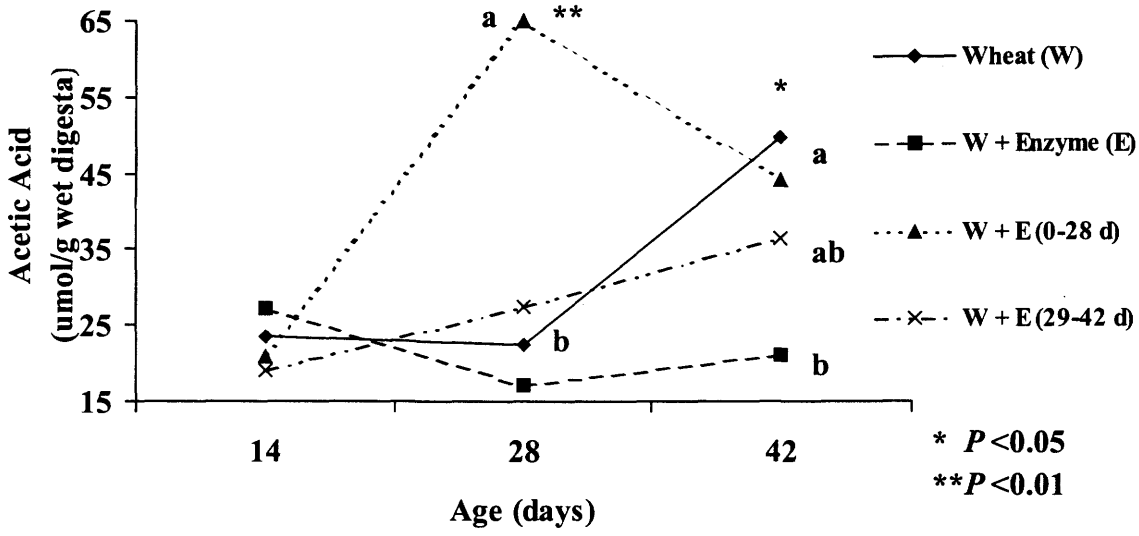

Figure 3.4.6. Effect of xylanase supplementation and age on caecal acetic acid in the wheat-fed broiler chicken in Experiment 2

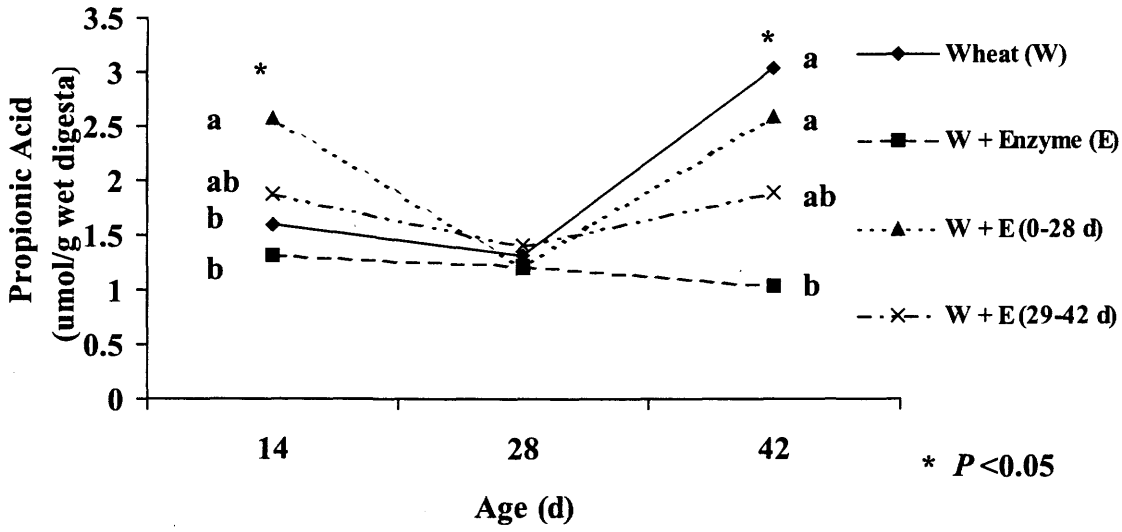

Figure 3.4.7. Effect of xylanase supplementation and age on caecal propionic acid in the wheat-fed broiler chicken in Experiment 2

42 d)) falling in between.

Caecal propionic acid (Figure 3.4.7) at 14 d was similar across treatments, except for the diet where enzyme was withdrawn at 28 d. This diet had higher caecal propionic acid levels. Caecal propionic acid levels are similar across treatments at 28 d. By 42 d, the treatments without enzyme supplementation have significantly higher levels of caecal propionic acid than the enzyme supplemented diet, with the late enzyme addition treatment falling in between.

Caecal iso-butyric acid (Figure 3.4.8) is again similar across treatments at 14 d, except for the late enzyme addition treatment, which has significantly higher levels. At 28 d, caecal iso-butyric levels are similar across treatments but are higher than 14 d levels. By 42 d, all treatments show decreased caecal iso-butyric acid levels, except the enzyme supplemented treatment, where caecal iso-butyric acid is significantly higher than the other treatments.

Total caecal VFAs (Figure 3.4.9) are similar across treatments at 14 d, and show significantly higher levels for the enzyme withdrawal diet at 28 d, when the enzyme is removed. By 42 d, the two unsupplemented treatments have significantly higher levels of caecal VFAs than the enzyme supplemented treatment, with the late enzyme addition treatment having intermediate levels, as seen with caecal propionic acid (Figure 3.4.7).

For the remaining VFAs (Table 3.4.9), no effect of dietary treatment was observed. The significant age effect in all cases was an increase in the level of each VFA with age of the bird.

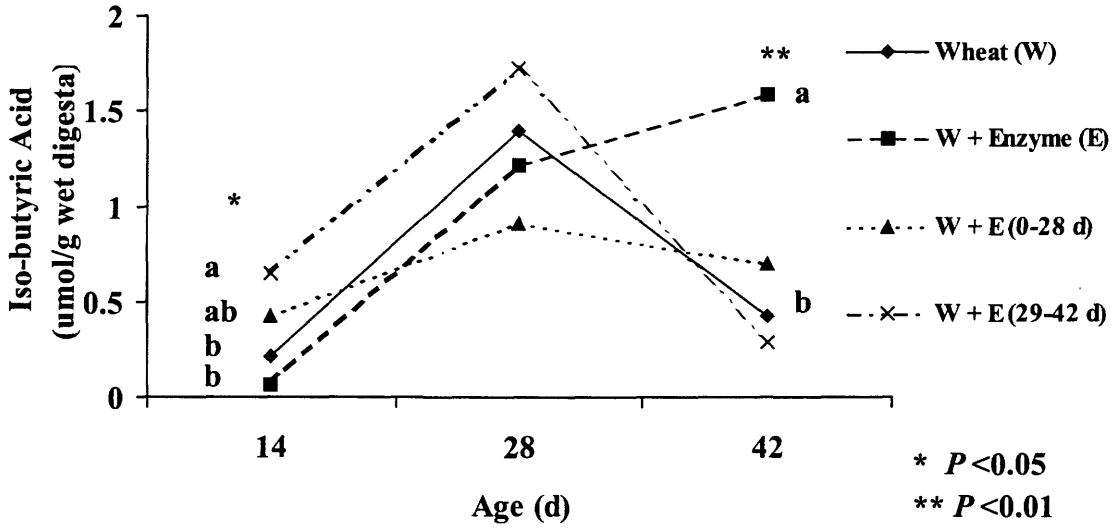

Figure 3.4.8. Effect of xylanase supplementation and age on caecal iso-butyric acid in the wheat-fed broiler chicken in Experiment 2

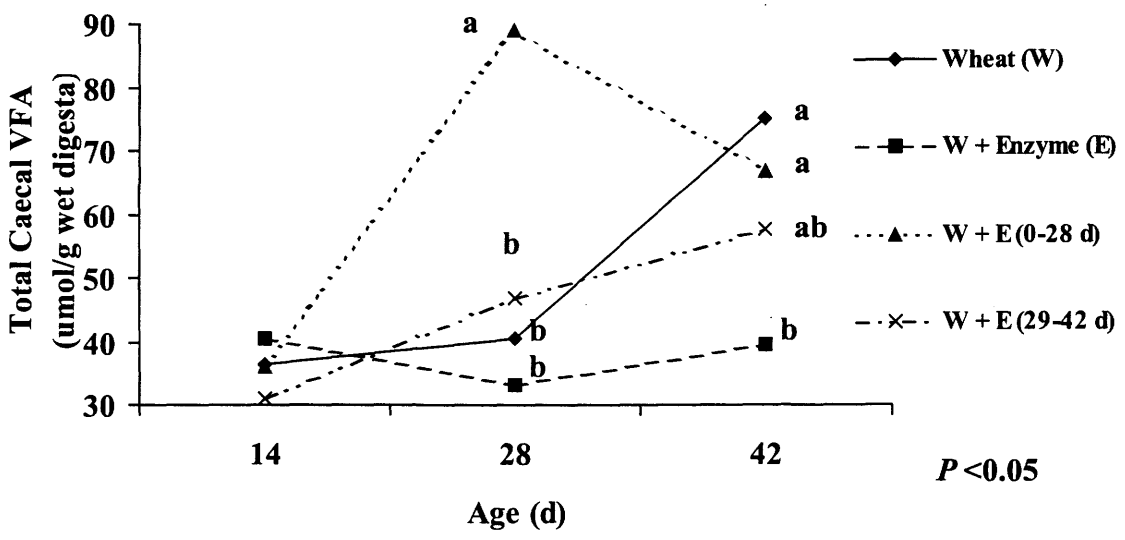

Figure 3.4.9. Effect of xylanase supplementation and age on total caecal volatile fatty acids in the wheat-fed broiler chicken in Experiment 2

3.5 Discussion and Conclusions

In both experiments, enzyme supplementation of wheat diets had a significant, positive effect on performance to 42 d over unsupplemented diets. From the results of the treatments where enzyme supplementation changed at 28 d it appears that late addition of enzyme may be as good as enzyme supplementation throughout the growing period, under certain conditions, as seen in Experiment 1. Early and continuous supplementation with enzyme, however, is more likely to guarantee a performance response if conditions are different, as seen in Experiment 2. Research has indicated that at younger ages (Veldman and Vahl, 1994; Steenfeldt *et al.*, 1998a) performance is improved largely as a result of improvements in viscosity reduction. As the bird ages, the improvements in performance are often even greater than at the younger ages despite viscosity reduction not being significant. Bedford and Morgan (1996) summarized 14 trials in which the relative improvement in feed conversion was greatest in 21-42 d old birds. It is suggested that the microflora are involved in the performance improvements later in the bird's life. Enzyme supplementation throughout the bird's life could, therefore, result in improved performance due to improvements in the size and structure of carbohydrate substrates, which may improve nutrient utilization by the bird through a reduction in competition for available substrates in the small intestine (Bedford, 1996b).

In Experiment 1 the birds were healthy and the diet included a growth promoting antibiotic, virginiamycin. In Experiment 2, the antibiotic was removed due to its known impact on gastrointestinal microflora and its absence may well have

altered the birds' responses to dietary treatments. In addition, an outbreak of J-virus occurred during the second experiment, which may have immune compromised the birds. Mortality was very high in Experiment 2 (14.3%) and despite the good performance of the surviving birds, health status may have had an impact on the GIT flora of all birds in this experiment.

In the current research, ileal and jejunal viscosities were elevated on the unsupplemented diets at 14 and 28 d of age but were not significantly different from enzyme supplemented birds by 42 d. The usefulness of the supplemented enzyme in reducing viscosity at later ages is not as obvious since intestinal viscosity decreases with age in broiler chickens (Petersen *et al.*, 1999). Whether the reduction in intestinal viscosity is actually an age effect or an acclimatization to the diet is still in question. The current experiment provides information in that regard, since the birds from whom enzyme was withdrawn at 28 d had numerically higher jejunal and ileal viscosities at 42 d of age than their fully supplemented counterparts. This suggests that diet acclimatization plays a role in GIT adaptation. The drop in digesta viscosity noted after 28 d for unsupplemented wheat fed birds in Experiment 2, shows adaptation is occurring on this diet. Since bacterial development in the GIT is related to digesta viscosity and available substrate, these changes are likely to relate to changes in bacterial numbers and fermentation.

Much of the published literature demonstrates that soluble pentosans, particularly those of high molecular weight (> 500 kDa) (Bedford and Classen, 1992), increase digesta viscosity in young broiler chicks (Choct and Annison, 1992b; Cowan, 1995; Morgan and Bedford, 1995; Van der Klis *et al.*, 1995). This increase

in digesta viscosity is thought to be one of the major factors in the anti-nutritive properties of wheat pentosans. It is suggested that the resultant proliferation of bacteria in the hindgut has a negative effect on digestion and absorption of nutrients (Choct *et al.*, 1992; Choct *et al.*, 1995; Choct *et al.*, 1996; Smits and Annison, 1996; Langhout, 1998). Very little research has been conducted specifically on the influence of digesta viscosity on bacterial numbers in the GIT. That which has been conducted has generally exaggerated the viscosity in the GIT to generate changes in the microflora by actually affecting passage rate (Dänicke *et al.*, 1997a, 1999). The viscosities obtained in those studies were, however, substantially higher (30-220 jejunum; 140-810 ileum) than those generated by the wheat diets used in the current studies (2.7-4.2 jejunum; 3.6-8.1 ileum). This would suggest that the model of high viscosity used in many research trials is not appropriate to the understanding of the effect of normal wheat diets on either the GIT microflora or nutrient utilization by the bird.

While the bacterial data in both current experiments are not conclusive, there is some indication that age and environmental conditions influence the bacterial response to enzyme treatment. In Experiment 1, where bacteria were cultured only at 42 d, there was a tendency for some ileal anaerobic bacteria to be slightly higher in number with enzyme supplementation than without. In the caeca, trends in the other direction are evident. At the same age in Experiment 2, ileal *Bifidobacterium* trends are more supportive of the literature in that the unsupplemented birds have slightly higher numbers in the ileum. The differences between this and the first experiment may be a result of the difference in antibiotic treatment between Experiments 1 and

2. Interactions between dietary enzyme use and the use of antibiotics have previously been reported. Antibiotic supplementation of enzyme diets was shown to impact enzyme response positively in one case of a virginiamycin and xylanase supplemented, wheat-based broiler diet (Schutte *et al.*, 1994), but negatively in a virginiamycin and mixed-enzyme supplemented, barley-based broiler diet (Elwinger and Teglöf, 1991) and a flavomycin and mixed-enzyme supplemented, barley-based layer diet (Vukic Vranjes and Wenk, 1996). Vukic Vranjes and Wenk (1996) suggested that the enzymic release of smaller sized NSPs for bacterial fermentation in birds with a mature gut flora results in increased VFA production and absorption, presumably in the ileum and caecum, and hence, improved energy utilization. Antibiotic supplementation, however, disrupts this flora, negating the beneficial effect of enzyme supplementation.

Caecal bacteria in Experiment 2, for the most part, are higher without enzyme than with and *Bacteroides* are significantly higher. This could be interpreted to indicate that the relatively lower viscosities in this research versus much of the published literature lead to substrate availability to the caecal bacteria without enzyme. Enzyme supplementation, in this case, appears to increase ileal fermentation leaving less substrate for caecal fermentation. This is opposite to much of the theory of ileal bacterial overgrowth on higher viscosity, NSP diets versus low NSP (ex. Corn) or enzyme-supplemented high NSP diets (Wagner and Thomas, 1978; Choct *et al.*, 1992; Choct *et al.*, 1995; Smits and Annison, 1996; Vahjen *et al.*, 1998; Dänicke *et al.*, 1999). The major difference between this and other

published studies is lower digesta viscosities in the current research, which could easily influence bacterial community composition (Choct *et al.*, 1996; Smits and Annison, 1996; Langhout, 1998).

On the whole, the use of bacterial culturing of a few genera of bacteria out of the many hundreds that exist in the poultry GIT was not as useful a procedure as anticipated. The reliance of bacteria growing in communities as complex as the GIT upon host tissue secretions and upon growth factors provided by other bacteria limits the effectiveness of traditional culturing of selected bacteria to observe responses to diet (Apajalahti and Bedford, 2000). The response of bacterial fermentation end-products, such as VFAs, to changes in diet and age may provide a more direct tool for analysis (Corrier *et al.*, 1990a; Choct *et al.*, 1996; Vahjen *et al.*, 1998; Choct *et al.*, 1999; Kocher *et al.*, 2000).

In Experiment 1, the only significant differences in VFA levels were between corn and wheat diets with corn resulting in lower ileal acetic acid, likely due to less fermentable substrate available to the bacteria in the ileum, and higher caecal propionic acid levels. The higher caecal propionic acid is likely due to the fermentation of residual starch in the corn (Van Soest, 1982) since ileal digestibility of the starch in corn has been shown to be as low as 85% (Noy and Sklan, 1995). It might also be due to fermentation of corn arabinoxylans which tend to favour the production of propionic acid (Lopez *et al.*, 1999). Little difference was observed in VFA levels in Experiment 1 between enzyme and unsupplemented wheat diets.

Data from diet by age interactions for ileal VFAs in Experiment 2, particularly propionic acid, unlike trends in the bacterial numbers, were highest with

enzyme at 42 d. This supports the suggestion by Vukic Vranjes and Wenk (1996) that mature birds have increased bacterial fermentation of enzyme released fibre substrates in the small intestine. At 14 and 28 d, however, ileal VFAs were highest without enzyme and lowest with enzyme, as discussed in the literature (Choct *et al.*, 1995; Choct *et al.*, 1996; Vahjen *et al.*, 1998). This suggests that the gut microflora adapt to the physicochemical conditions in the gastrointestinal tract with age and that bacterial fermentation patterns may reflect this better than numbers of the selected bacterial types measured in this study.

GIT bacterial adaptation appears to occur both with age and dietary change. Support for the diet side of this observation may be found in data from the treatments where enzyme supplementation changed at 28 d. These birds showed rapid responses in both viscosity and VFA levels to changes made at 28 d. This was seen since the logistics of the experiment required the diets to be changed after the body weights were measured each period. Sampling, however, took from that point in time until later in the afternoon due to the large number of birds in the experiments. Thus, the viscosity effects in birds fed the changed diets were observed within hours of the dietary changes. The birds from whom enzyme was withdrawn had increased levels of ileal acetic acid as well as significantly increased levels of caecal acetic, butyric and total VFAs, indicating increased fermentation of NSP substrates in both the ileum, where a minor increase in viscosity occurred, and in the caecum, into which the soluble NSP substrates could subsequently enter. In contrast, the birds receiving enzyme supplementation at 28 d had lowered ileal acetic acid and total VFA levels whereas caecal VFA levels were not significantly affected by enzyme

addition, indicating a possible change in the activity of the ileal flora initially adapted to larger molecular weight NSP substrates, but now being exposed to smaller ones.

By 42 d, when viscosities were more similar between treatments, this effect appears reversed with the enzyme withdrawal birds in both experiments showing signs of decreased ileal fermentation as compared to enzyme supplemented levels. Ileal acetic acid at 42 d was somewhat lowered by enzyme withdrawal in Experiment 1 and ileal acetic, propionic and total VFAs were lowered in Experiment 2. By 42 d the birds receiving late enzyme addition (28 d) increased ileal fermentation as shown by significantly higher levels of acetic acid than any other treatment and total ileal VFAs as high as fully supplemented birds in Experiment 2 and by numerically highest ileal acetic acid levels in Experiment 1. Caecal fermentation patterns by 42 d showed all VFAs except iso-butyrate higher in enzyme withdrawal birds in both experiments. Since iso-butyrate is highest in the caecum on enzyme supplemented diets and is a product of protein fermentation it is possible that the enzyme, by producing smaller, soluble NSP oligomers that could enter the caecum, helped release proteins bound in cell wall components of wheat as suggested by Choct *et al.* (1996). Other known sources of iso-butyrate production in the caeca are from uric acid degradation (Braun and Campbell, 1989) and possibly endogenous and/or microbial protein breakdown. Karasawa (1989) concluded that caecal amino acids may be derived from diet, urine and endogenous proteins such as microbes, sloughed intestinal mucosa and digestive enzymes, but does not evaluate specific VFA production from these protein sources. Uric acid degradation is unlikely to have been influenced by the dietary differences whereas endogenous, particularly microbial

protein quantities, could have been impacted by enzyme supplementation.

The observed changes in fermentation pattern, both over time and with change in enzyme status, suggest that while viscosity is high enough at the younger ages to alter the GIT environment such that anaerobic bacteria proliferate in the ileum of wheat-fed birds, by 42 d this effect is reversed and enzyme supplementation actually enhances ileal bacterial fermentation. In particular, the bacterial population of unsupplemented birds grew while they weren't on enzyme, then enzyme supplementation at 28 d provided these bacteria with additional, easily degradable substrate and a similar viscosity, thereby increasing ileal fermentation, as indicated by the VFA levels for this treatment at 42 d. Apajalahti and Bedford (1998) suggest that the ileal flora adapt to the presence of smaller xylo-oligomers generated by enzyme hydrolysis which provide preferential substrate to certain bacteria of the ileum. Since the bacteria do adapt, it is possible, then, that the mature (42 d) ileal flora may be different enough that it is better able to handle the xylo-oligomers in unsupplemented wheat diets than the potentially immature 28 d flora.

The size of the GIT influences overall bacterial numbers and, consequently, the overall fermentive capacity of the GIT. While no significant differences in GIT size were evident between enzyme supplemented and unsupplemented wheat-fed birds in either experiment, there was a difference in GIT size between corn and wheat diets (Experiment 1). This difference in GIT size is a partial explanation for the differences in VFA production or fermentation capacity seen between corn and wheat fed birds. The GIT is also substantially larger in 42 d versus 28 d birds with a larger surface area for mucosal bacterial attachment and, therefore, may have

resulted in the increased levels of total ileal VFAs from fermentation of enzyme-generated xylo-oligomers in enzyme supplemented birds observed in Experiment 2.

While the literature suggests that caecal fermentation increases with enzyme supplementation due to increased availability of easily degradable substrate to the caecal bacteria (Choct *et al.*, 1995; 1996; Bedford 1996b; Choct *et al.*, 1999), in the current experiments greater fermentation occured in the caeca without enzyme supplementation. The diets used in the current studies yielded viscosities much lower than other published studies and, therefore, unsupplemented diets in these experiments resulted in degradation of soluble arabinoxylans lower in the hindgut and release of these substrates to the caecal bacteria for fermentation. Enzyme supplementation of the diets in the current studies resulted in the release of soluble substrates higher in the GIT, and, therefore, resulted in ileal fermentation of these substrates. This is particularly evident in older birds with more mature bacterial populations that allow less substrate to enter the caeca with enzyme supplementation than without. A comparison of the amount of NSP remaining in the terminal ileum between enzyme supplemented and unsupplemented birds would be useful in determining how much NSP is degraded by bacteria and, ultimately, how much benefit the bird is getting from the VFAs produced.

Most of the literature deals with birds from 7-21 d of age (Choct *et al.*, 1995; Choct *et al.*, 1996; Langhout, 1998; Vahjen *et al.*, 1998; Dänicke *et al.*, 1999). In the current experiments bacterial culturing was done at 28 and 42 d in consideration of the observations made by Barnes *et al.* (1972) that it takes about 6 weeks to establish an adult flora in the caecum. While Salanitro *et al.* (1978) observed

dramatic changes in the GIT flora of corn/soy fed birds as young as 14 d of age, the published literature to date does not address the adaptation seen in this study in the GIT flora of birds on wheat diets, with and without enzyme supplementation, between 28 and 42 d of age. From the data in Experiment 1, it is clear that the GIT environment is very different between corn and wheat-based diets and this will have an impact on the development of the GIT bacteria. There is a lack of literature on the development of GIT bacteria with age in domestic poultry and, particularly little on wheat-fed birds. In addition, the differentiation between luminal and mucosally attached bacteria may have been beneficial in more accurately correlating the VFA responses with the bacterial population changes in this study.

In conclusion, the current study has demonstrated that the GIT itself adapts more to the source of NSP rather than to its chemical structure since the wheat diets in Experiment 1, whether supplemented with enzyme or not, yielded larger GIT components than the corn diet. The GIT flora adapt to the conditions created by the presence of NSP in the diet, both to the viscosity changes by increasing in number with increased viscosity and to substrate availability with specific bacteria and overall fermentation, in some areas of the tract, increasing in response to the enzyme-generated NSP substrates in the GIT. Volatile fatty acids can be used as a measure of bacterial activity and are responsive to changes in diet NSP levels as well as to the adaptation in the microflora that occur with age. It is suggested that the age-related changes in bacterial development interact with the substrates provided in the GIT by enzyme supplementation to yield the changes in fermentation seen in the ileum of older, wheat-fed broilers.

4.0 INFLUENCE OF DIETARY NON-STARCH POLYSACCHARIDE AND ENDOXYLANASE SUPPLEMENTATION ON OVERALL LEVELS OF AEROBIC AND ANAEROBIC BACTERIA IN THE ILEUM AND CAECUM OF THE BROILER CHICKEN AT TWO AGES.

4.1 Abstract

An experiment was conducted to determine the impact of dietary NSP and endoxylanase supplementation on aerobic and anaerobic bacteria in the hindgut of broiler chickens. A total of 600 male broiler chicks were assigned at one d of age to 4 replicates each of 3 dietary treatments. A wheat-based diet with or without added endoxylanase (Avizyme 1300, 1 kg/tonne) was compared to a corn-based diet with a similar nutrient composition. Bird performance was measured at 14, 28 and 42 d of age. Birds were sampled at 28 and 42 d, and ileal and caecal samples were cultured for total aerobes and total anaerobes. Corn and enzyme supplemented wheat-fed birds performed equally well with unsupplemented wheat-fed birds having the highest gain to feed ratios after 14 d and overall. Enzyme supplementation of wheat diets resulted in the highest numbers of caecal anaerobes with corn-fed birds having the lowest number. At 42 d, birds had higher numbers of caecal anaerobes than at 28 d. At 28 d, caecal aerobes were highest on enzyme-supplemented wheat diets ($P<0.10$) while at 42 d, caecal anaerobes were lowest on the corn diet and similar for

the two wheat diets. Therefore, despite performance similarities between corn-fed and enzyme-supplemented, wheat-fed birds, there are definite differences in the bacteria present in the hindgut on each diet. This is likely due to the difference in residual dietary substrate in the hindgut of the birds fed different diets and its ability to enter the caeca. The substrates present in the ileum of enzyme-supplemented birds may be of benefit to both the bird, by being more easily digested, and to the different cross-section of caecal bacteria present. Less NSP substrate is likely to be available in the hindgut of corn-fed birds.

4.2 Introduction

The use of enzymes to improve the performance of wheat-fed broilers is common practice in the poultry industry. The concern that dietary NSP, such as that found in wheat, causes elevation of digesta viscosity and subsequent increases in the population of anaerobic bacteria in the small intestine is raised in a number of research papers (Annison *et al.*, 1968; Wagner and Thomas, 1978; Choct *et al.*, 1995; Choct *et al.*, 1996; Smits *et al.*, 1998; Langhout, 1998; Choct *et al.*, 1999; Langhout *et al.*, 1999). Enzyme use, while reducing digesta viscosity, is also suspected of influencing the native bacterial populations in the ileum and caecum of broiler chickens (Choct *et al.*, 1995; Choct *et al.*, 1996; Bedford 1996b; Vahjen *et al.*, 1998; Choct *et al.*, 1999). Research has indicated a probable negative role of bacterial overgrowth in terms of competition for substrates and decreased nutrient digestibility (Bedford, 1996a; Langhout *et al.*, 2000) as well as possible positive roles such as improved gut health and exclusion of colonization by pathogens

(Stavric *et al.*, 1992; Nisbet *et al.*, 1993; Corrier *et al.*, 1995; Hume *et al.*, 1995; Bedford 1996b). A previous study indicated a shift in bacterial populations with dietary NSP, as expected, and with age (Chapter 3). That study demonstrated that certain anaerobic bacteria increase fermentation with increased viscosity and that ileal anaerobic bacteria increase fermentation with increasing substrate availability from enzyme-generated NSP. Nearly all published literature on GIT microflora as influenced by NSP and enzyme refers to birds from 0-21 d of age. The previous study showed changes occurring at 28 d of age and later, meaning that work with young birds cannot be extrapolated to older birds. Since that study showed only moderate bacterial response from the small number of groups cultured, the current study was designed to look at broader groups of bacteria to see if some of the observed trends could be confirmed. The objective of the current study was, therefore, to investigate changes in total aerobic and anaerobic bacteria numbers in both the ileum and caecum of the broiler chicken fed diets differing in NSP content and enzyme supplementation at two ages.

4.3 Materials and Methods

4.3.1 Bird Management, Diets and Sampling

A total of 600 male, day old broiler chickens (Hubbard x Petersen) were randomly assigned to four replicate pens each of three dietary treatments. Floor pens bedded with straw accommodated 50 birds each. Dietary treatments included a corn-based diet, a wheat based diet, and a wheat based diet supplemented with a commercial xylanase (Avizyme 1300: Xylanase activity 2700 IU/g, Protease activity

800 IU/g; Finnfeeds International, Marlborough, Wiltshire, UK, SN8 1XN). Diets were calculated to be of similar nutrient composition with 3,000 kcal/kg AME and 22 %, 20 % and 19 % CP, respectively for the starter, grower and finisher diets (Chapter 3, Table 3.3.1a,b,c). Virginiamycin was not added in an attempt to minimize any impact other than the dietary treatments on the GIT flora. Feed and water were provided *ad libitum*.

Birds were cared for using standard management practices of the University of Saskatchewan. Initial room temperature was 35 C and was gradually decreased to 22 C by 35 d. This temperature was maintained to the end of the trial. The starter diet was fed from 0-14 d, the grower from 15-28 d and the finisher from 29-42 d. Pen weights were taken at each diet change. Feeders were weighed to calculate feed consumption. Weight gain and feed conversion efficiency were calculated for each two week period and overall.

At 28 and 42 d, two birds per replicate were killed by injection of T-61 euthanasia solution (Embutramide 200mg/mL, Hoechst Roussel Vet Canada Inc., Regina, SK). The abdominal cavities of the birds were opened and the terminal 10cm of the ileum and the left caecum were tied off with dental floss, ligated and placed immediately on ice. These samples were taken to the laboratory for microbiological plating on selective media.

The experimental protocol was approved by the Animal Care Committee, and the procedures were performed in accordance with the requirements of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

4.3.2 Bacteriology

Samples of ileal and caecal contents were weighed into sterile, conical dilution tubes with peptone water and cysteine hydrochloride in a laminar flow hood. Samples were serial diluted with a solution of peptone water and cysteine hydrochloride. Plating was done using an Autoplate 4000 (Spiral Biotech, Bethesda, MD) spiral diluter. The medium in the plates was TSA Blood Agar Base (DIFCO Laboratories, Detroit, MI) with 5% sheeps blood. Plates for total aerobes were incubated for 24 h at 37 C under aerobic conditions and plates for total anaerobes were incubated for 24 h at 37 C in anaerobic jars with BBL GasPak Plus (Becton Dickinson Microbiology Systems, Cockeysville, MD) anaerobic system envelopes with palladium catalyst. Anaerobic indicators (BBL GasPak Disposable Anaerobic Indicators, Becton Dickinson Microbiology Systems, Sparks, MD) were placed in the jars prior to sealing. Colonies on incubated plates were counted by hand using the Spiral Biotech sector counting grid.

4.3.3 Statistical Analysis

The experiment was analysed as a two-way analysis of variance using the general linear models procedure of SAS (SAS Institute, 1989) to determine significant effects of treatment, age and interactions. Significant mean differences ($P<0.05$) were determined using Duncan's multiple range test (Steel and Torrie, 1980). The bacterial data was log transformed prior to statistical analysis and predetermined orthogonal contrasts were used to compare corn and wheat as well as enzyme and unsupplemented wheat diets.

4.4 Results

4.4.1 Performance

The corn-fed birds grew the fastest early on (0-28 d) but were the same size as wheat fed birds by 42 d. Birds fed unsupplemented wheat diets ate more feed over the course of the experiment and consequently had poorer feed conversion than either the corn-fed birds or the enzyme-supplemented, wheat-fed birds (Table 4.4.1). There were no treatment effects on bird mortality.

4.4.2 Bacteriology

Main effects of diet and age on bacterial numbers showed that only caecal anaerobes were affected by dietary treatment with birds on the corn diet having the lowest number and those on the enzyme-supplemented wheat diet having the highest number ($P<0.06$) (Table 4.4.2). The unsupplemented wheat diet resulted in numbers of caecal anaerobes intermediate to the other two diets. In addition, 42 d birds had higher numbers of caecal anaerobes than 28 d birds.

When analysed by age, the only significant treatment effect on bacterial populations was that of wheat-fed birds at 42 d having significantly greater numbers of caecal anaerobic bacteria than corn-fed birds (Figure 4.4.1). The only trend of note was at 28 d when enzyme-supplemented wheat-fed birds had higher numbers of aerobes ($P=0.10$) in the caeca. This trend was gone by 42 d.

Table 4.4.1 Effect of dietary NSP and xylanase supplementation on performance of broiler chickens at three ages

Parameter and Period	Treatments			*P*	SEM
	Corn	Wheat	Wheat + Enzyme		
Wt. Gain					
0 – 14 d	0.347[a]	0.333[b]	0.334[b]	*	0.003
15-28 d	1.045[a]	1.000[b]	0.992[b]	***	0.008
29-42 d	1.279	1.259	1.232	NS	0.018
0 – 42 d	2.670	2.592	2.558	NS	0.024
Feed Cons.					
0 – 14 d	0.403	0.471	0.429	NS	0.008
15-28 d	1.617[b]	1.832[a]	1.612[b]	**	0.036
29-42 d	2.978	3.135	2.869	NS	0.058
0 - 42 d	5.541[b]	6.013[a]	5.577[b]	0.07	0.096
Gain:Feed[1]					
0 – 14 d	0.923	0.866	0.852	NS	0.016
15-28 d	0.712[a]	0.605[b]	0.678[a]	***	0.015
29-42 d	0.488[a]	0.458[b]	0.490[a]	***	0.005
0 - 42 d	0.614[a]	0.555[b]	0.601[a]	***	0.008

[1]Mortality corrected.
[a,b] Means within rows with no common superscript differ significantly (*=$P \leq 0.05$;**=$P \leq 0.01$;***=$P \leq 0.001$; NS = not significant).
SEM = pooled standard error of the mean.

Table 4.4.2 Main effects of dietary NSP, xylanase supplementation and age on numbers of total aerobes and total anaerobes in the ileum and caecum of broiler chickens

Variables		Bacterial Counts (log_{10}CFU/g wet digesta)			
		Ileal Aerobes	Ileal Anaerobes	Caecal Aerobes	Caecal Anaerobes
Diet	Corn	6.95	7.08	7.53	8.32^{b}
	Wheat	6.63	7.16	7.63	8.72^{ab}
	Wheat plus Enzyme	6.55	6.87	7.92	9.09^{a}
Age (d)	28	6.81	7.05	7.72	8.44^{b}
	42	6.56	7.03	7.66	8.99^{a}
Statistics					
Diet		NS	NS	NS	0.06
Age		NS	NS	NS	*
Diet X Age		NS	NS	NS	NS
Contrasts:					
Corn vs Wheat (No E)		NS	NS	NS	NS
Wheat (No E) vs Wheat plus Enzyme		NS	NS	NS	NS
SEM		0.156	0.148	0.108	0.147

[a,b] Means, within a column and main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; NS = not significant).
SEM = pooled standard error of the mean.

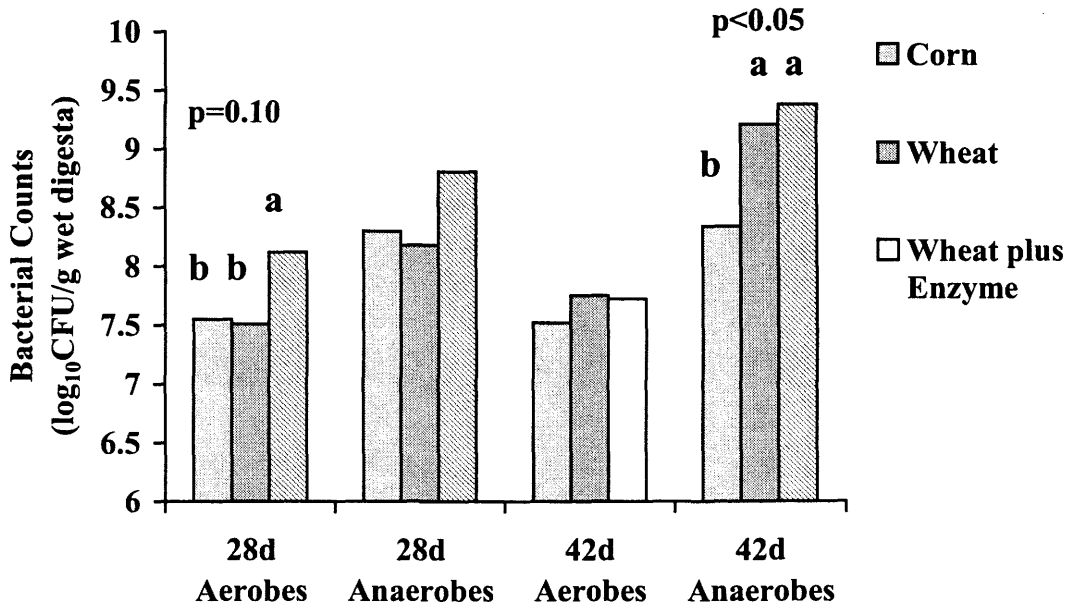

Figure 4.4.1. Effect of dietary NSP and xylanase supplementation on caecal aerobes and caecal anaerobes

4.5 Discussion and Conclusions

The significantly poorer performance of the birds fed wheat diets without enzyme supplementation might lead to speculation that ileal anaerobic bacterial overgrowth is occurring. A limited number of studies (Feighner and Dashkevicz, 1988; Bedford, 1996a; Choct *et al.*, 1996; Langhout *et al.*, 2000) have linked poorer performance of birds fed diets high in NSP with increased ileal bacterial proliferation. Others have shown that increased bacterial fermentation in the ileum of birds fed high NSP diets (from either wheat pentosans or highly methylated citrus pectin) has a negative impact on nutrient digestion and absorption (Choct *et al.*, 1992; Choct *et al.*, 1999; Langhout *et al.*, 2000). Improvements in bird performance occur when rye or wheat diets are supplemented with a xylanase or NSP-degrading enzyme (Grootwassink *et al.*, 1989; Choct *et al.*, 1996). Improvements in nutrient digestibility also occur with enzyme supplementation (Langhout *et al.*, 1997; Choct *et al.*, 1999). In the current study, however, ileal bacteria measured as total aerobes and total anaerobes, were not affected by dietary treatment, despite the improvements in performance seen with both enzyme supplementation and the corn-based diets.

While enzyme supplementation of the wheat diet resulted in significantly ($P<0.06$) greater numbers of caecal anaerobes than the corn diet and a trend toward higher numbers of caecal aerobes, it is necessary to analyze the data by age to observe the trends more closely. The enzyme diet appears to result in the highest numbers of both aerobes and anaerobes in the caeca at 28 d, while both wheat diets have higher numbers of anaerobes by 42 d. By 42 d, aerobe numbers are similar

across treatments. It may be that at the low digesta viscosities generated by the wheat diets used in the current study, as seen from the data of previous studies (Chapter 3, Tables 3.4.2 and 3.4.3), enzyme supplementation provides substrate readily accessible to the caecal bacteria. At 28 d both aerobes and anaerobes flourish in the caeca then, as the ileal flora matures and adapts to degrade the larger NSP on the unsupplemented wheat diets, they produce higher levels of lactate (Vahjen *et al.*, 1998) which provide the caecal anaerobes with substrate, allowing for their proliferation. A similar concept was proposed by Bedford (1996b) for enzyme supplemented diets and was said to lead to better GIT health and, potentially, better bird performance. In the current experiment, by 42 d, both supplemented and unsupplemented wheat diets resulted in similar levels of caecal anaerobes, likely due to the absence of ileal viscosity differences at this age. Performance, however, is still better on the enzyme supplemented diet which may be due to the differences in substrate utilization described by Bedford (1996b) and Vahjen *et al.* (1998).

In addition, although performance was similar between the corn-fed and the enzyme-supplemented, wheat-fed birds, the bacterial profiles were different. This may be accounted for by differences in the residual dietary substrates present in the GIT between the diets. The corn diet has a lower inherent level of NSP than either wheat diet due to the NSP content of the major grains (Choct and Annison, 1990). Bedford and Classen (1992) observed that both the molecular weight distribution of residual NSP and the actual sugar composition of the NSP in the broiler GIT changes with differing levels of dietary rye substitution for wheat and differing levels of pentosanase supplementation. Further evidence for the presence of different

substrates may be found in the work of Vahjen *et al.* (1998) who found that xylanase-supplementation of wheat-based diets resulted in lower levels of enterobacteria and gram-positive cocci but higher levels of tissue-associated *Lactobacillus* spp. in the duodenum, jejunum and ileum of broilers up to 21 d of age. The differences in the types and quantities of fermentation end-products in the GIT, such as lactate and VFAs, measured by these researchers are also indicative of differences in substrate availability. Apajalahti and Bedford (2000) have also shown differences in the composition of the community of GIT bacteria in broiler chickens fed wheat, rye and corn-based diets which were said to be accounted for by the differences in the substrate available to the bacteria for fermentation. While these differences did not affect performance between the corn-fed birds and the enzyme supplemented, wheat-fed birds, they may have contributed to the poorer performance of the unsupplemented, wheat-fed birds. In addition, using total bacterial numbers may also cover up possible beneficial or negative effects of specific genera of bacteria.

Dietary substrate also has an influence on overall numbers of bacteria in the ileum of broiler chickens. Bacterial numbers have been shown to be higher on high NSP diets by a number of researchers (Wagner and Thomas, 1978; Langhout, 1998; Smits *et al.*, 1998; Langhout *et al.*, 1999). Indirect evidence for increased bacterial numbers in the ileum of high NSP diets through the demonstration of increased VFA production has also been published (Annison *et al.*, 1968; Choct *et al.*, 1996; Jørgensen *et al.*, 1996; Choct *et al.*, 1999). In the current study, overall numbers of ileal and caecal aerobes and anaerobes were used to determine a general effect of

dietary treatment on the intestinal bacteria. The differences observed in anaerobic bacterial numbers could have an influence on performance parameters, depending upon the types of bacteria that increase. Some anaerobic bacteria are beneficial and can enhance performance, while others may be detrimental. Ideally, determination of the specific genera of anaerobic bacteria that change with dietary treatment should be determined but the culturing of selected specific genera of bacteria in previous experiments (Chapter 3) was not adequate to demonstrate overall dietary differences.

In conclusion, despite performance similarities between corn-fed and enzyme-supplemented, wheat-fed birds, there are differences in the numbers of bacteria present in the caecum of birds on each diet. This is likely due to the difference in residual dietary substrate in the hindgut of the birds fed different diets. The substrates present in the ileum of enzyme-supplemented birds may be of benefit to both the bird, possibly through use of the end-products of bacterial fermentation, and possibly simply by providing an environment conducive to the proliferation of beneficial bacteria, and to the caecal bacteria present. Less NSP substrate is likely to be available in the hindgut of corn-fed birds. The unsupplemented wheat-fed birds likely performed poorly as a result of the time required for the bacteria of the ileum to adapt to utilizing the NSP substrates and, ultimately, providing breakdown products to the caecal bacteria.

5.0 INFLUENCE OF AGE, DIETARY NON-STARCH POLYSACCHARIDES AND ENDOXYLANASE SUPPLEMENTATION ON THE GASTROINTESTINAL TRACT, BACTERIAL FERMENTATION AND RESIDUAL NSP SUBSTRATES IN THE BROILER CHICKEN.

5.1 Abstract

An experiment was conducted to determine the impact of age, NSP content and xylanase supplementation on gastrointestinal tract size, bacterial fermentation, and residual NSP in the broiler chicken. Male broilers (840) were fed diets containing corn (C), wheat (W) or wheat supplemented with endoxylanase (E, Avizyme 1300, 1 kg/t). Birds were sampled weekly for GIT lengths and weights and collection of GIT contents for viscosity, volatile fatty acid (VFA) and residual NSP (total sugar) determination. Jejunal viscosity was highest ($P \leq 0.05$) for the W treatment at all ages except 7 d. Ileal viscosity was highest in this diet at all ages. Jejunal and ileal lengths and weights as a proportion of body weight were generally largest for W, followed by E, and smallest for C. Caecal lengths followed the same pattern but caecal weights were highest for E, followed by W then C. Ileal VFAs were not affected by treatment. Caecal acetic acid was highest for birds fed the W and E treatments, whereas caecal pH, isobutyric, isovaleric and valeric acids were highest for corn-fed

birds. Caecal propionic acid was highest for birds fed C, followed by W, with E having the lowest levels. There was a significant interaction between diet and age for propionic acid. The higher NSP content of the wheat diets likely caused the increased GIT size of the W and E treatments. While the NSP in W and E treatments are broken down by the caecal bacteria to acetate and butyrate, the C treatment resulted in the production of propionate and isovalerate. The latter finding suggests that undigested starch and protein from corn enter the caeca and are being fermented by bacteria in this location. E diets had higher amounts of soluble and low mol. wt. NSP derived arabinose and xylose present in ileal contents ($P \leq 0.001$) than W and C diets. As the birds aged, proportionally more arabinose and xylose was solubilized from the W diet. The E treatment had higher, but relatively steady levels of soluble arabinose and xylose whereas the C treatment had the lowest levels and no change was seen with age. This suggests a bacterial adaptation to wheat NSP with age of the bird and the presence of NSP resistant to ileal bacterial hydrolysis in corn diets.

5.2 Introduction

The poultry feeding industry has been using enzymes to supplement wheat-based diets for a number of years. Research on enzyme modes of action has shifted from non-starch polysaccharide (NSP) breakdown, decreased digesta viscosity and increased availability of or access to nutrients (Chesson, 1987; Petterson and Åman, 1989; Bedford and Classen, 1992; van der Klis *et al.*, 1995; Steenfeldt *et al.*, 1998a,b), towards the interaction between the effects of decreased digesta viscosity and the gastrointestinal tract (GIT) microflora (Choct *et al.*, 1996; Vahjen *et al.*,

1998; Dänicke *et al.*, 1999). Previous experiments in this area (Chapters 3 & 4) have demonstrated the adaptability of the microflora to the presence of dietary NSP and xylanase supplementation of wheat-based diets. These experiments also indicated a possible age-related change in bacterial development that appeared to involve an interaction with the substrates provided in the GIT by enzyme supplementation and bacterial fermentation.

Intact NSP from wheat have been shown to increase digesta viscosity in broiler chickens (Annison, 1993; Chesson, 1995; Choct and Annison, 1992b; Choct *et al.*, 1996). With enzyme supplementation, NSP are depolymerized into smaller molecular weight components (Petterson and Åman, 1989; Bedford *et al.*, 1991; Bedford and Classen, 1992; Bedford and Apajalahti, 2001). Some studies have demonstrated the poorer absorption of D-xylose and L-arabinose, relative to D-glucose by chickens (Longstaff *et al.*, 1988; Schutte, 1990; Schutte *et al.*, 1991) and in pigs (Yule and Fuller, 1992; Haberer *et al.*, 1998) therefore increasing their availability as substrate for microbial breakdown. The release of monosaccharides from NSP, however, is not the major, immediate effect of enzyme supplementation, depolymerization is. While the depolymerization of NSP is of benefit in terms of reduced digesta viscosity and improved performance, it is, as yet, unclear what the fate of these NSP in the chicken GIT is.

The objectives of this study were to examine the respective influences of age, dietary NSP and endoxylanase supplementation on the size of the GIT, bacterial fermentation within the GIT and on the size, volume and composition of residual NSP substrates in the terminal ileum of the broiler chicken.

5.3 Materials and Methods

5.3.1 Bird Management and Diets

A total of 840 day-old male commercial (Peterson X Arbor Acres) broiler chicks were randomly assigned to 4 replicate pens for each of 3 dietary treatments. Dietary treatments consisted of practically formulated starter, grower and finisher rations based on corn, wheat or wheat plus enzyme (Avizyme 1300; xylanase activity 2700 IU/g; protease activity 800 IU/g; Finnfeeds International, Marlborough, Wiltshire, UK, SN8 1XN) at the recommended supplementation level of 0.1 % of the diet. The diets were calculated to provide 3000 kcal/kg energy and 22 %, 21 % and 20 % crude protein for the starter, grower and finisher diets, respectively (Chapter 3, Table 3.3.1). Virginiamycin was not added in an attempt to minimize any impact other than the dietary treatments on the GIT flora. Feed and water were provided *ad libitum*. Diets contained Celite® (Celite Corp., Lompac, CA 93436) as an acid-insoluble ash marker. Diets were changed at 14 and 28 d of age with the starter in crumble form and the grower and finisher diets pelleted. The high viscosity wheat used to make the diets was not available for the finisher diets and so a commercial feed grade wheat was used for this phase. *In vitro* viscosity of all diets was measured according to the method of Bedford and Classen (1993).

Birds were cared for using standard management practices of the University of Saskatchewan. Initial room temperature was 35 C and was gradually decreased to 22 C by 35 d. This temperature was maintained to the end of the trial. Pen weights were taken at each diet change. Feeders were weighed to calculate feed

consumption. Mortality was recorded daily along with weights of dead birds. Gain to feed ratio was calculated and corrected for mortality weight gain.

The experimental protocol was approved by the Animal Care Committee, and the procedures were performed in accordance with the requirements of the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

5.3.2 Bird Sampling and Gastrointestinal Tract Measures

Four birds per replicate were killed each week by injection with T-61 euthanasia solution (Embutramide 200mg/mL, Hoechst Roussel Vet Canada Inc., Regina, SK). Intestinal tracts were excised and divided into the jejunum, ileum and paired caeca. Component lengths, and weights were taken before and after the components were gently rolled to extract their contents. The contents were pooled across the 4 birds and analyzed for pH, viscosity and volatile fatty acids. The pH was measured immediately upon pooling using a portable pH meter (Model 59002-00, Cole-Parmer Instrument Co., Niles, IL). Viscosity was measured on supernatant from jejunal and ileal contents using a Brookfield Viscometer (Model DV-III, Brookfield Engineering Laboratories, Inc., Stoughton, MA). The remaining samples were frozen for subsequent VFA analysis. Samples from the terminal ileum (halfway from the Meckel's Diverticulum to the ileo-caecal junction) of 4 additional birds per replicate were taken for determination of dry matter, acid-insoluble ash and residual NSP (total sugar analysis).

5.3.3 Volatile Fatty Acid Analysis

Sub-samples of jejunal (0.5 g), ileal (0.5 g) and caecal contents (0.2 g) were weighed into microcentrifuge tubes, vortexed with 1 ml of prepared internal standard (iso-caproic acid) solution, and centrifuged at 15, 900 x *g*. Volatile fatty acids were measured on the supernatant using a gas chromatograph (Varian Star 3400Cx equipped with a Varian 8200Cx autosampler, Varian, Walnut Creek, CA). The column used was a glass capillary column packed with carbowax fused silica (Stabilwax-DAS, RESTEK Corporation, Bellefonte, PA). The injector temperature was 220 C, the initial column temperature was 140 C, held for 5 minutes, then gradually increased to 220 C, and the flame ionization detector temperature was 230 C. Results were expressed as mmol/L and converted to μmol/g wet digesta. The method used was a modification of Corrier *et al.* (1990a) with the modifications being adaptations for use with a capillary column and different internal standards, as outlined above.

5.3.3.1 Non-starch Polysaccharide Analysis and Digestibility Determination

Ileal samples were taken as whole wet samples (200 mg), as soluble ileal digesta (supernatant from 500 mg ileal digesta centrifuged at 15, 900 x *g*), or as the low molecular weight portion of the soluble ileal digesta (500 μL of supernatant filtered through Nanosep^{TM} microconcentrators (Pall Filtron Corporation, Northborough, MA) with a molecular weight cut-off of 100 kDa. The filtration was conducted in a high-speed centrifuge (13,800 x *g*). All three fractions were subjected to partial NSP analysis (Englyst and Hudson, 1987; Englyst, 1989) for quantification

of the total sugar content of the ileal digesta. The removal of starch was eliminated from the NSP analysis to prevent loss of smaller oligosaccharides and free sugars present in the ileal digesta which would have been present as a result of either microbial or host enzyme degradation of dietary NSP. The analysis, therefore, commenced with acid hydrolysis of the component NSP, followed by derivitization to alditol acetates and quantification by gas chromatography.

Whole ileal samples were also subjected to dry matter (AOAC, 1990) and acid-insoluble ash analyses. Diets and digesta were analyzed for acid insoluble ash marker using a modification of the method of Vogtmann *et al.* (1975). Samples (1-2 g) were weighed into 16x125 mm disposable borosilicate tubes, ashed at 500 C for 24 h or until contents were reduced to white ash. This was followed by slowly adding 5 ml of 4N HCl and vortexing, covering the tubes with glass marbles and heating in an oven at 120 C for one h before centrifuging at 2500 × *g* for 10 min. The supernatant was then removed and samples washed repeatedly with 5 ml water (with vortexing and centrifugation as described above). Samples were then dried at 80 C overnight, followed by ashing at 500 C overnight. The percent acid insoluble ash was calculated as (total ashed wt - tube wt) / (original - tube wt). The acid-insoluble ash marker was used to calculate dry matter and NSP (or total sugar) digestibility.

5.3.4 Statistical Analysis

The experiment was analyzed as a two-way analysis of variance using the general linear models procedure of SAS (SAS Institute, 1989) to determine

significant effects of treatment, age and interactions. Significant mean differences ($P \leq 0.05$) were determined using Duncan's multiple range test (Steel and Torrie, 1980).

5.4 Results

5.4.1 Bird Performance and Digesta Viscosity

All birds performed well with no treatment differences in gain, except at 14 d where corn-fed birds had higher gain than the two wheat-fed treatments (Table 5.4.1). Feed consumption, at 14 d, was highest for the wheat-fed birds, lowest for the corn-fed birds, and intermediate for the enzyme-supplemented, wheat-fed birds. There were no treatment differences at any other age. Gain to feed ratio was again only affected by treatment at 14 d where corn-fed birds outperformed both wheat-fed treatments. This trend was evident across all ages. There were no treatment effects on bird mortality.

Digesta viscosities were lowest for birds fed the corn and enzyme-supplemented wheat diets at most ages (Table 5.4.2) except at 35 d where both jejunal and ileal viscosity were similar between the two wheat-based diets, and at 42 d where the corn treatment was lowest, the wheat plus enzyme treatment intermediate, and the wheat treatment was highest. There was no effect of diet on ileal digesta dry matter content (Table 5.4.3). Age, however, did significantly influence ileal dry matter with 28 and 35 d birds having higher ileal dry matter content than 42 d birds. Analysis of diet *in vitro* viscosity showed that all

Table 5.4.1 Effect of dietary NSP and enzyme supplementation on body weight gain, feed consumption and gain to feed ratio in broiler chickens

Parameter and Period	Treatments			*P*	SEM
	Corn	Wheat	Wheat plus Enzyme		
Weight Gain					
0 – 14 d	0.356[a]	0.328[b]	0.332[b]	**	0.005
15–28 d	1.007	1.008	1.008	NS	0.003
29–42 d	1.265	1.364	1.272	NS	0.048
0 – 42 d	2.627	2.700	2.612	NS	0.050
Feed Consumption					
0 – 14 d	0.569[b]	0.600[a]	0.590[ab]	*	0.006
15–28 d	1.393	1.425	1.455	NS	0.032
29–42 d	4.494	4.389	4.411	NS	0.096
0 - 42 d	9.190	8.552	8.984	NS	0.218
Gain:Feed[1]					
0 – 14 d	0.766[a]	0.678[b]	0.691[b]	***	0.012
15–28 d	0.965	0.932	0.919	NS	0.023
29–42 d	0.508	0.494	0.482	NS	0.008
0 - 42 d	0.703	0.658	0.660	NS	0.010

[1]Mortality corrected.

[a,b] Means within rows with no common superscript differ significantly (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

three starter diets had similar, low viscosities with the corn diet having the lowest extract viscosity in the grower and finisher phases (Table 5.4.4). The highest viscosity was seen in the unsupplemented wheat grower diet. The enzyme had a significant effect on *in vitro* viscosity only in the grower diets.

5.4.2 Gastrointestinal Tract Measures

A common trend was seen for ileal and jejunal measurements as a proportion of body weight (Table 5.4.5). The ranking of sizes was wheat > wheat plus enzyme > corn, although the significance of these differences varied. Values for corn-fed birds were consistently smaller than for those fed wheat while the wheat plus enzyme treatment values were intermediate. A similar ranking was seen for caecal length, but in contrast, caecal weight measurements were largest for the wheat plus enzyme treatment followed by the wheat and corn treatments, in diminishing order. A similar order was found for caecal content weight. For all measures, the relative size decreased with age of the bird.

Significant diet by age interactions were seen for both full and empty jejunal weights, and empty caecal weights. In the jejunum, the wheat diet yielded heavier weights from 0-21 d after which the enzyme supplemented diet resulted in equally heavy jejunal weights with corn diets having the lightest components (Table 5.4.6). In the caecum, at 14 and 21 d, the enzyme diet resulted in the heaviest empty weights with wheat and corn being similar until 35 d when enzyme and corn treatments yielded the heaviest weights and unsupplemented wheat resulted in lighter weights.

Table 5.4.2 Effects of diet and age on jejunal and ileal digesta supernatant viscosity (cps) in broiler chickens

Gut Segment	Age (d)	Treatments			*P*	SEM
		Corn	Wheat	Wheat plus Enzyme		
Jejunum	7	1.98	2.31	2.13	NS	0.086
	14	1.91^{b}	4.09^{a}	2.35^{ab}	*	0.403
	21	1.84^{b}	6.79^{a}	3.12^{b}	***	0.671
	28	1.77^{b}	6.29^{a}	2.79^{b}	***	0.624
	35	2.14^{b}	2.98^{a}	2.59^{a}	**	0.126
	42	1.85^{c}	3.32^{a}	2.58^{b}	***	0.197
Ileum	7	2.08^{b}	3.16^{a}	2.31^{ab}	0.08	0.213
	14	2.11^{b}	5.12^{a}	2.80^{b}	**	0.478
	21	2.38^{b}	16.45^{a}	4.95^{b}	**	2.278
	28	3.08^{b}	8.53^{a}	3.78^{b}	**	0.856
	35	2.83^{b}	3.69^{ab}	4.46^{a}	0.08	0.306
	42	2.55^{b}	4.51^{a}	2.82^{b}	*	0.339

^{a,b,c} Means within rows with no common superscript differ significantly (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).
SEM = pooled standard error of the mean.

Table 5.4.3 Effect of diet and age on ileal digesta dry matter content in broiler chickens

Variables		Ileal Digesta Dry Matter Content (%)
Diet	Corn	28.78
	Wheat	29.63
	Wheat plus Enzyme	26.35
Age (d)	7	27.68[ab]
	14	28.22[ab]
	21	26.53[ab]
	28	32.90[a]
	35	31.31[a]
	42	22.88[b]
Statistics		
	Diet	NS
	Age	*
	Diet X Age	NS
	SEM	0.92

[a,b] Means within a column, within a main effect, with no common superscript differ significantly

(* = $P \leq 0.05$; ** = $P \leq 0.01$; NS = not significant).

SEM = pooled standard error of the mean.

Table 5.4.4 Effect of major dietary grain on *in vitro* extract viscosity (cps) of diets

Period	Treatments			*P*	SEM
	Corn	Wheat	Wheat plus Enzyme		
Starter	2.50	2.79	2.85	NS	0.07
Grower	2.35[c]	3.80[a]	2.96[b]	***	0.19
Finisher	2.14[b]	2.88[a]	3.21[a]	***	0.15

[a,b,c] Means within rows with no common superscript differ significantly (*** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

Table 5.4.5 Main effects of diet and age on size and fill of the gastrointestinal tract

Variables		Gastrointestinal Tract Size and Fill (as a proportion of body weight)[1]											
		Jejunum				Ileum				Caeca			
		Length	Full Wt	Empty Wt	Contents	Length	Full Wt	Empty Wt	Contents	Length	Full Wt	Empty Wt	Contents
Diet	Corn	9.56^{b}	3.22^{b}	1.93^{b}	1.30^{b}	9.40^{b}	2.62^{c}	1.43^{b}	1.19^{b}	3.80^{b}	0.73^{b}	0.46^{b}	0.27
	Wheat	10.19^{a}	3.46^{a}	2.04^{a}	1.42^{a}	10.12^{a}	2.93^{a}	1.57^{a}	1.36^{a}	4.09^{a}	0.77^{ab}	0.47^{b}	0.30
	Wheat plus Enzyme	9.84^{ab}	3.30^{b}	1.97^{b}	1.33^{ab}	9.76^{ab}	2.77^{b}	1.53^{a}	1.24^{b}	4.01^{a}	0.82^{a}	0.49^{a}	0.32
Age (d)	7	27.10^{a}	5.13^{a}	3.02^{a}	2.12^{a}	26.57^{a}	4.67^{a}	2.47^{a}	2.19^{a}	10.56^{a}	1.20^{a}	0.69^{a}	0.52^{a}
	14	13.50^{b}	3.77^{b}	2.35^{b}	1.42^{b}	12.90^{b}	2.97^{b}	1.71^{b}	1.26^{b}	5.07^{b}	0.78^{b}	0.53^{b}	0.25^{b}
	21	7.54^{c}	3.34^{c}	1.86^{c}	1.48^{b}	7.67^{c}	2.72^{c}	1.39^{c}	1.33^{b}	3.03^{c}	0.70^{bc}	0.44^{c}	0.27^{b}
	28	4.72^{d}	2.86^{d}	1.76^{d}	1.11^{c}	4.95^{d}	2.31^{d}	1.25^{d}	1.06^{c}	2.20^{d}	0.70^{bc}	0.42^{cd}	0.28^{b}
	35	3.60^{e}	2.57^{e}	1.53^{e}	1.04^{cd}	3.70^{e}	2.05^{e}	1.12^{e}	0.92^{d}	1.65^{e}	0.64^{cd}	0.39^{de}	0.25^{b}
	42	2.72^{f}	2.29^{f}	1.37^{f}	0.92^{d}	2.79^{f}	1.91^{e}	1.11^{e}	0.81^{d}	1.30^{f}	0.60^{d}	0.38^{e}	0.23^{b}
Statistics													
Diet		**	**	**	*	**	***	***	***	**	**	**	0.06
Age		***	***	***	***	***	***	***	***	***	***	***	***
Diet x Age		NS	**	*	NS	NS	NS	NS	NS	NS	NS	*	NS
SEM		0.508	0.063	0.036	0.031	0.493	0.061	0.031	0.032	0.191	0.017	0.008	0.011

[1](value/body weight) X 100.

[a-f] Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; ** = $P \leq 0.001$; NS = not significant); SEM = pooled standard error of the mean.

5.4.3 Volatile Fatty Acid Analysis

Volatile fatty acid data indicate very little dietary treatment influence on jejunal and ileal volatile fatty acid levels (Table 5.4.7). Corn diets yielded the lowest levels of jejunal isovaleric acid while corn and enzyme-supplemented diets yielded the lowest levels of jejunal valeric and ileal isobutyric acids. Unsupplemented wheat diets resulted in the highest levels of these VFAs. Ileal pH was highest for the corn diet and similar for the two wheat diets. In the jejunum, pH was higher at 7 and 14 d compared to later in the experiment. Jejunal VFA levels all peaked at 21 d whereas in the ileum, VFAs were highest at 7 d. The pH of the ileum was lowest at 14 d and highest at 42 d. There were no significant interactions whereas caecal pH, isobutyric, isovaleric and valeric acids were all significantly higher for birds fed the corn than either wheat diet. Only with propionic acid did enzyme supplemented birds differ from both corn and wheat-fed birds with corn diets resulting in the highest propionic acid levels, unsupplemented wheat diets being intermediate and enzyme supplemented diets yielding the lowest level of propionic acid. Age had no effect on caecal pH but did influence VFA level. For acetic, propionic, butyric, valeric and total caecal VFAs, levels peaked at 28 d. For isobutyric and isovaleric, levels were also numerically highest at 28 d. For all individual VFAs, levels were lowest at 7 d with 14 d levels being either statistically similar or slightly higher than 7 d levels. The diet by age interaction was only significant for propionic acid and it is shown in Figure 5.4.1. Propionic acid level for enzyme supplemented wheat diets

Table 5.4.6 Effect of age and dietary treatment on weights of selected gastrointestinal tract components (as a percentage of bird body weight)

GIT Measure[1]	Age (d)	Treatments			*P*	SEM
		Corn	Wheat	Wheat plus Enzyme		
Full	7	5.06	5.23	5.11	NS	0.080
Jejunal	14	3.88^{a}	4.00^{a}	3.43^{b}	*	0.090
Weight	21	3.28^{ab}	3.58^{a}	3.17^{b}	*	0.072
	28	2.66	2.95	2.98	0.09	0.067
	35	2.27^{b}	2.62^{a}	2.83^{a}	***	0.061
	42	2.19	2.39	2.28	NS	0.048
Empty	7	3.03	3.09	2.94	NS	0.048
Jejunal	14	2.38^{ab}	2.47^{a}	2.20^{b}	0.06	0.048
Weight	21	1.83	1.95	1.79	NS	0.036
	28	1.64^{b}	1.81^{a}	1.82^{a}	*	0.030
	35	1.42^{b}	1.52^{b}	1.67^{a}	**	0.031
	42	1.26^{b}	1.43^{a}	1.41^{a}	**	0.025
Empty	7	0.69	0.71	0.66	NS	0.016
Caecal	14	0.51^{b}	0.52^{ab}	0.58^{a}	*	0.012
Weight	21	0.39^{b}	0.44^{ab}	0.48^{a}	**	0.011
	28	0.40	0.41	0.44	NS	0.126
	35	0.41^{a}	0.35^{b}	0.41^{a}	*	0.012
	42	0.35	0.37	0.40	0.09	0.010

[1](value/body weight) X 100.

[a,b] Means within rows with no common superscript differ significantly (* = $P \leq 0.05$; ** = $P \leq 0.01$; NS = not significant).

SEM = pooled standard error of the mean.

Table 5.4.7 Effect of age and dietary treatment on pH and volatile fatty acid levels (µmol/g wet digesta) in the jejunal and ileal digesta of broiler chickens

		Jejunum						Ileum					
		pH	Acetic	Propionic	Iso-valeric	Valeric	Total VFA	pH	Acetic	Propionic	Iso-butyric	Iso-valeric	Total VFA
Diet	Corn	6.07	11.90	0.96	0.45^{b}	0.07^{b}	13.54	7.21^{a}	11.05	0.74	0.000^{b}	0.24	12.13
	Wheat	6.09	12.26	1.05	0.56^{a}	0.14^{a}	14.19	6.97^{b}	9.95	0.69	0.026^{a}	0.26	11.01
	Wheat plus Enzyme	6.12	12.00	1.00	0.53^{a}	0.10^{ab}	13.79	6.85^{b}	11.83	0.84	0.003^{b}	0.29	13.06
Age (d)	7	6.14^{ab}	10.95^{b}	0.84^{b}	0.57^{ab}	0.09^{bc}	12.62^{b}	7.16^{ab}	12.79	0.95^{a}	0.04^{a}	0.41^{a}	14.29
	14	6.26^{a}	12.51^{b}	1.04^{b}	0.49^{bcd}	0.04^{c}	14.26^{b}	6.73^{c}	10.28	0.65^{bc}	0.00^{b}	0.31^{ab}	11.35
	21	6.03^{b}	14.91^{a}	1.30^{a}	0.66^{a}	0.21^{a}	17.24^{a}	6.88^{bc}	11.59	0.90^{ab}	0.01^{ab}	0.15^{b}	12.73
	28	6.00^{b}	11.90^{b}	0.96^{b}	0.54^{bc}	0.13^{b}	13.78^{b}	7.01^{abc}	11.54	0.81^{ab}	0.01^{ab}	0.29^{ab}	12.76
	35	6.07^{b}	11.07^{b}	0.97^{b}	0.40^{d}	0.11^{bc}	12.65^{b}	7.01^{abc}	10.44	0.75^{abc}	0.00^{b}	0.26^{ab}	11.50
	42	6.05^{b}	10.97^{b}	0.89^{b}	0.43^{cd}	0.06^{bc}	12.50^{b}	7.27^{a}	9.07	0.50^{c}	0.00^{b}	0.18^{b}	9.83
Statistics													
Diet		NS	NS	NS	*	*	NS	**	NS	NS	**	NS	NS
Age		*	***	**	***	***	***	*	0.10	**	*	**	0.08
Diet X Age		0.09	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEM		0.027	0.319	0.037	0.019	0.012	0.368	0.052	0.408	0.039	0.004	0.022	0.461

[a-d] Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant). SEM = pooled standard error of the mean.

Table 5.4.8 Effect of diet and age on pH and volatile fatty acid levels (µmol/g wet digesta) in the caecum of broiler chickens

Variables		Caeca							
		pH	Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFA
Diet	Corn	5.88[a]	78.22[b]	6.77[a]	0.70[a]	27.63	1.19[a]	1.61[a]	116.12
	Wheat	5.69[b]	86.19[a]	4.09[b]	0.53[b]	25.07	0.88[b]	1.14[b]	117.90
	Wheat plus Enzyme	5.57[b]	85.29[a]	3.37[c]	0.47[b]	27.11	0.92[b]	1.08[b]	118.25
Age (d)	7	5.83	65.88[d]	2.52[e]	0.67	19.22[c]	1.06	0.11[d]	89.47 d
	14	5.57	75.98[c]	3.51[d]	0.44	17.79[c]	1.05	0.69[c]	99.46[d]
	21	5.73	93.21[ab]	5.91[b]	0.53	28.08[b]	0.98	1.41[b]	130.11[b]
	28	5.78	98.48[a]	6.91[a]	0.68	38.60[a]	1.05	1.95[a]	147.65[a]
	35	5.78	84.78[bc]	4.81[c]	0.53	30.04[b]	0.85	1.79[a]	122.81[bc]
	42	5.58	81.09[c]	4.79[c]	0.55	25.88[b]	0.99	1.72[a]	115.02[c]
Statistics									
Diet		**	*	***	**	NS	***	***	NS
Age		NS	***	***	NS	***	NS	***	***
Diet X Age		NS	NS	***	NS	NS	NS	NS	NS
SEM		0.04	1.92	0.30	0.03	1.10	0.03	0.09	2.95

[a-e]Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

from 14 through 42 d of age. It was also higher than the unsupplemented wheat diet from 14 d but at 28 d the propionic acid level from this diet peaked at a high value before decreasing back to the level of the enzyme diet for the remainder of the production cycle. At the peak, the propionic acid level was not different than for the corn treatment.

5.4.4 Ileal Residual NSP Analysis

Residual NSP in the digesta of the terminal ileum were affected by diet (Table 5.4.9). Total arabinose and xylose were highest in ileal samples from enzyme supplemented diets with unsupplemented wheat diets having levels of residual arabinose that were similar to corn diets but levels of residual xylose that were significantly higher than corn. Mannose followed a similar pattern to xylose while galactose was highest in corn-fed birds, intermediate in the enzyme birds, and lowest in the unsupplemented birds. Residual glucose was significantly higher in birds fed both wheat diets than those fed corn. Residual arabinose and xylose were highest at 42 d while residual glucose was highest at 28 d. The interaction means, shown for arabinose and xylose only in Figure 5.4.2, indicate that except at 14 d where the wheat diet shows a dramatic increase in the presence of residual arabinose and xylose in the terminal ileum, all diets result in similar levels of residual arabinose with xylose being somewhat lower from the corn diet.

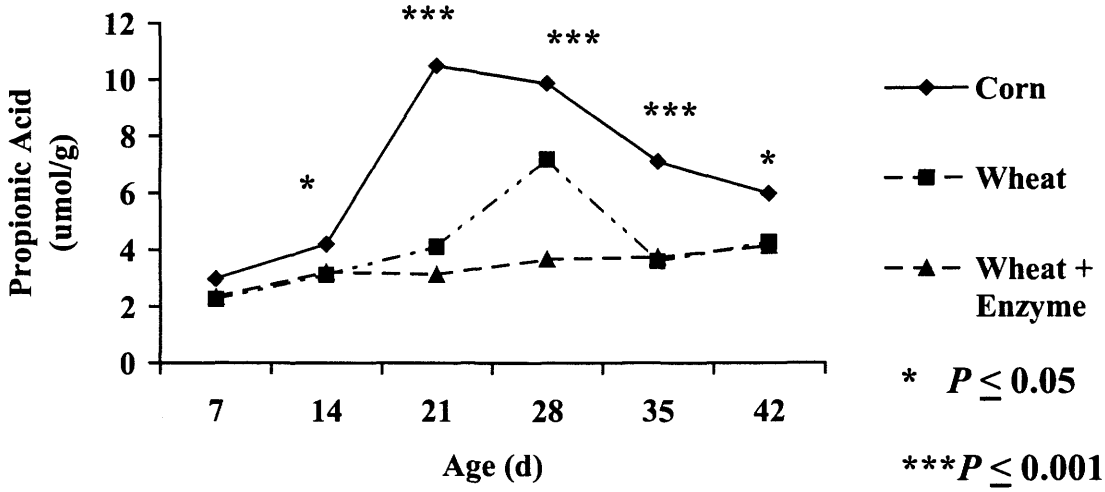

Figure 5.4.1 Effect of diet and age on caecal propionic acid levels in the broiler chicken

Table 5.4.9 Effect of diet and age on residual NSPs in terminal ileal digesta

Variables		Total NSPs (% of Dry Matter)				
		Arabinose	Xylose	Mannose	Galactose	Glucose
Diet	Corn	6.26^{b}	6.13^{c}	0.98^{c}	7.94^{a}	8.07^{b}
	Wheat	6.07^{b}	7.63^{b}	1.21^{b}	5.22^{c}	14.82^{a}
	Wheat plus Enzyme	7.14^{a}	8.88^{a}	1.43^{a}	6.45^{b}	16.12^{a}
Age (d)	7	5.43^{b}	5.88^{c}	1.08	6.37	10.38^{bc}
	14	6.65^{b}	7.21^{bc}	1.33	7.35	9.31^{c}
	21	6.28^{b}	6.81^{bc}	1.27	5.97	14.61^{b}
	28	5.91^{b}	6.84^{bc}	1.43	6.93	20.38^{a}
	35	6.16^{b}	7.77^{b}	1.01	5.66	10.69^{bc}
	42	8.51^{a}	10.81^{a}	1.13	6.95	12.64^{bc}
Statistics						
Diet		*	***	***	***	***
Age		***	***	0.06	NS	***
Diet X Age		NS	NS	NS	NS	*
SEM		0.21	0.31	0.05	0.24	0.86

$^{a-c}$Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

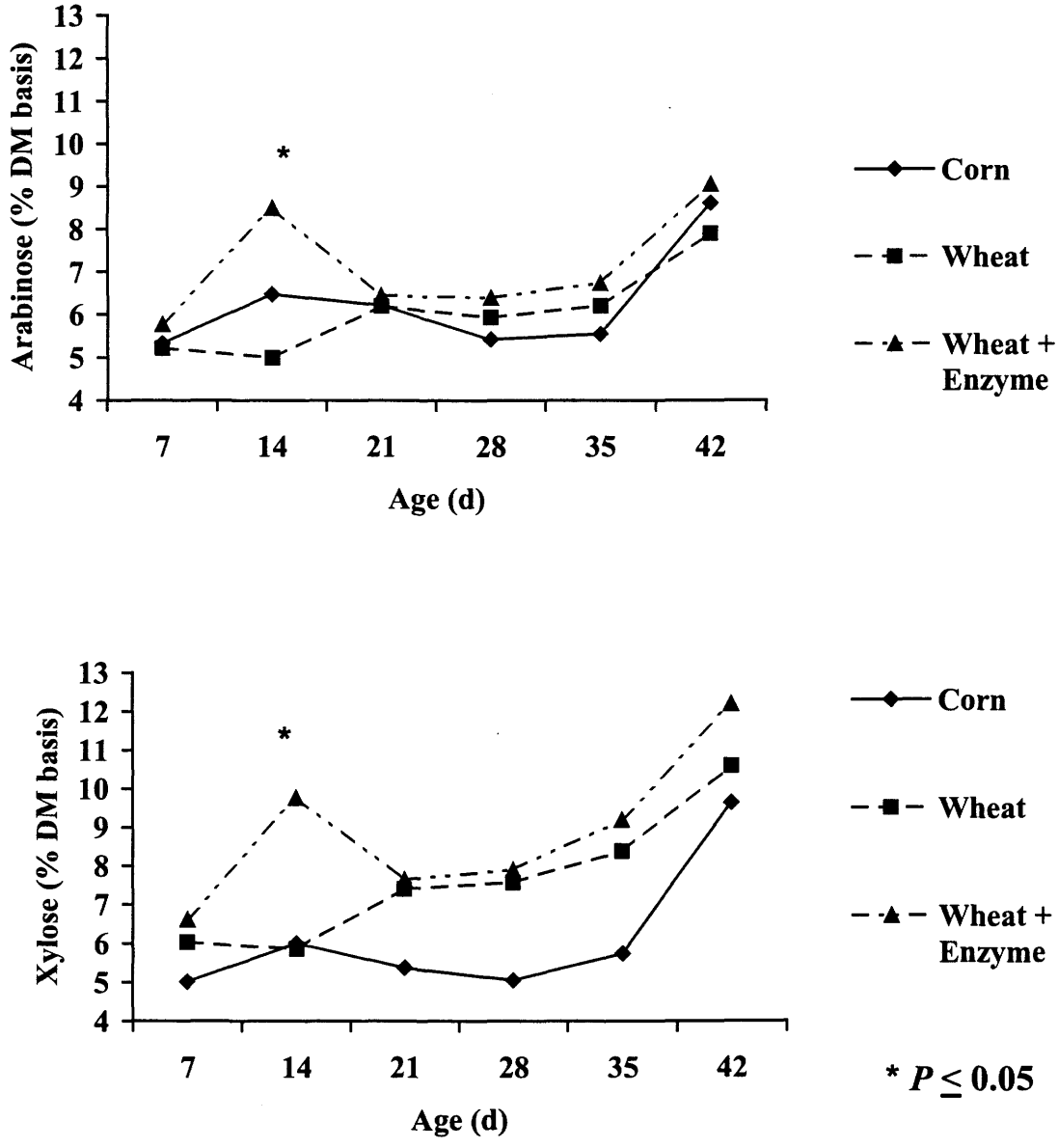

Figure 5.4.2 Effect of diet and age on total arabinose and xylose content of the terminal ileal digesta of broiler chickens

For soluble residual NSP, enzyme diets had the highest residual arabinose and xylose, followed by wheat, with corn diets yielding the lowest soluble arabinose and xylose levels (Table 5.4.10). Soluble mannose residues were highest in birds fed both wheat diets versus corn, while soluble glucose residues in the ileum of enzyme-supplemented birds were similar to unsupplemented wheat-fed birds but significantly higher than for corn-fed birds. Soluble arabinose residues were higher at 42 d than at 7-28 d while soluble xylose residues at 42 d were only significantly higher than 7 d levels. No other soluble residual sugars were affected by age. Interaction means for soluble arabinose and xylose (Figure 5.4.3) indicate that while both sugars have high levels at all ages from the enzyme diet and low levels from the corn diet, the levels of soluble arabinose and xylose for the unsupplemented wheat diet increase with age of the bird.

The analysis of the low molecular weight, soluble, residual NSP shows that arabinose, xylose and mannose are highest in the ileum of enzyme-supplemented birds with wheat and corn-fed birds being similar (Table 5.4.11). Galactose is highest for corn-fed birds with the two wheat diets being similar. Age only affects residual arabinose and xylose levels with the levels of low molecular weight, soluble arabinose and xylose increasing steadily with age of the bird. The significant interaction means, shown in Figure 5.4.4, indicate that this is primarily due to the enzyme supplemented wheat diet.

Table 5.4.10 Effect of diet and age on residual soluble NSPs from the supernatant from the terminal ileal digesta of broiler chickens

Variables		Soluble NSPs (% of Dry Matter)				
		Arabinose	Xylose	Mannose	Galactose	Glucose
Diet	Corn	0.59^{c}	0.33^{c}	0.74^{b}	3.18	4.42^{b}
	Wheat	1.04^{b}	1.28^{b}	0.96^{a}	2.47	5.81^{ab}
	Wheat plus Enzyme	1.50^{a}	1.97^{a}	1.07^{a}	2.85	6.88^{a}
Age (d)	7	0.77^{b}	0.80^{b}	0.79	2.47	5.98^{ab}
	14	1.02^{b}	1.18^{ab}	0.97	2.95	3.83^{c}
	21	0.97^{b}	1.15^{ab}	1.10	3.03	6.73^{a}
	28	1.00^{b}	1.23^{ab}	1.00	2.94	7.31^{a}
	35	1.10^{ab}	1.24^{ab}	0.73	2.40	4.29^{c}
	42	1.41^{a}	1.56^{a}	0.96	3.20	6.09^{ab}
Statistics						
Diet		***	***	*	0.08	**
Age		*	*	NS	NS	**
Diet X Age		NS	NS	NS	NS	NS
SEM		0.07	0.10	0.04	0.12	0.34

$^{a-c}$Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).
SEM = pooled standard error of the mean.

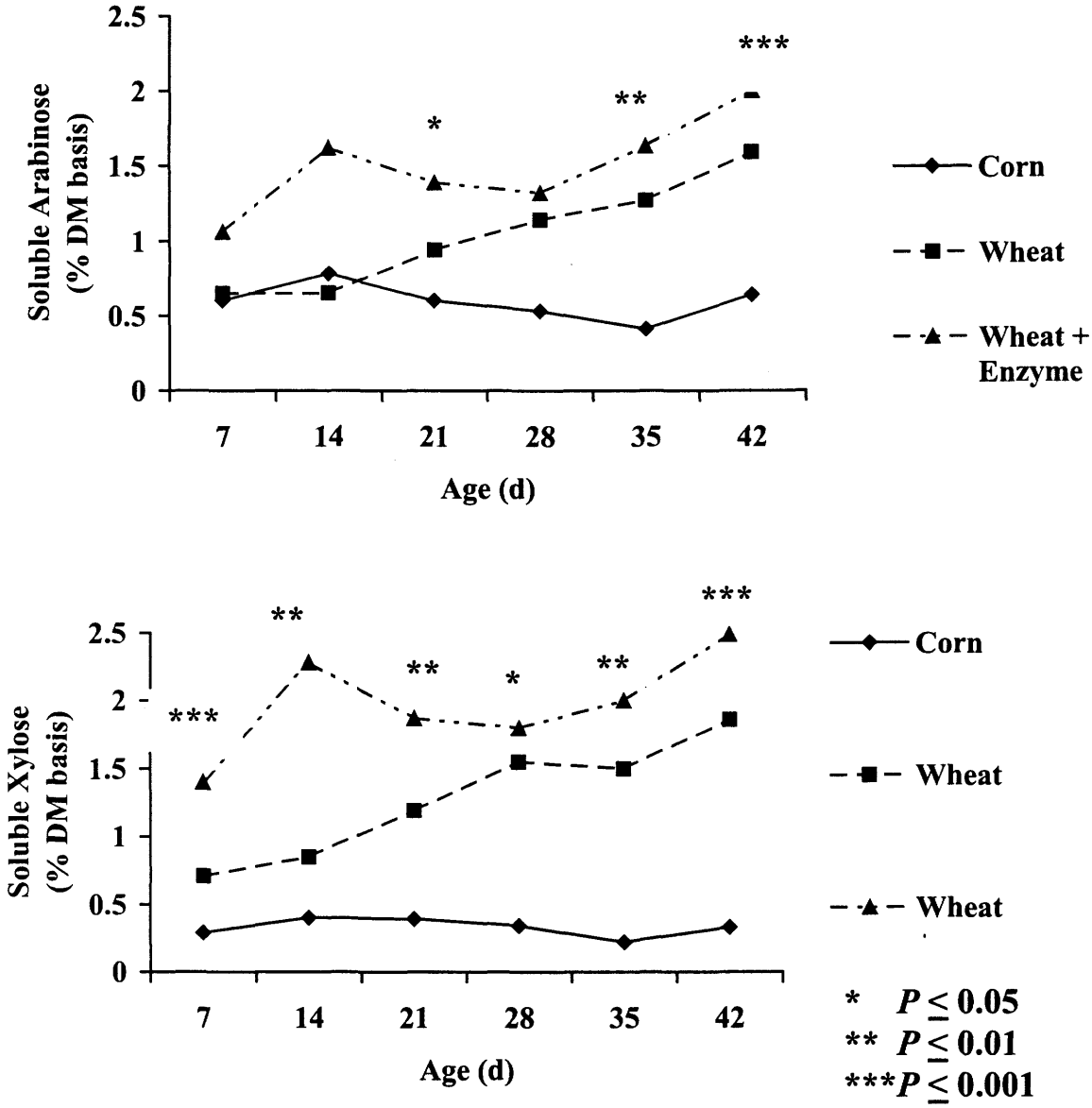

Figure 5.4.3 Effect of diet and age on the residual soluble arabinose and xylose in the terminal ileal digesta of broiler chickens

Table 5.4.11 Effect of diet and age on residual low molecular weight (<100 kDa), soluble NSPs from the filtered supernatant from the terminal ileal digesta of broiler chickens

Variables		Low Molecular Weight, Soluble NSPs (% of Dry Matter)				
		Arabinose	Xylose	Mannose	Galactose	Glucose
Diet	Corn	0.22^{b}	0.14^{b}	0.64^{b}	2.69^{a}	5.27
	Wheat	0.20^{b}	0.26^{b}	0.63^{b}	1.73^{b}	5.27
	Wheat plus Enzyme	0.50^{a}	0.80^{a}	0.79^{a}	2.15^{b}	6.34
Age (d)	7	0.15^{c}	0.16^{c}	0.59	1.72	5.10
	14	0.28^{bc}	0.36^{b}	0.74	2.34	6.17
	21	0.28^{bc}	0.39^{b}	0.73	2.18	5.76
	28	0.29^{bc}	0.40^{ab}	0.69	2.09	5.73
	35	0.38^{ab}	0.47^{ab}	0.59	2.21	4.50
	42	0.45^{a}	0.60^{a}	0.76	2.61	6.50
Statistics						
Diet		***	***	*	***	NS
Age		**	**	NS	NS	NS
Diet X Age		*	*	NS	NS	NS
SEM		0.03	0.05	0.03	0.10	0.27

[a-c]Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

Arabinose to xylose ratio was affected by diet in all fractions analyzed (Table 5.4.12). The corn diet always provided the highest ratio with the two wheat diets being similar in the whole ileal contents but with the enzyme-supplemented diet resulting in a significantly lower arabinose to xylose ratio in the soluble and low molecular weight soluble fractions of ileal contents.

The interaction (Figure 5.4.5a) shows that for corn diets, there is an increase in arabinose to xylose ratio at 21 d, followed by a decrease. Interestingly, in soluble ileal digesta supernatant, there is a different pattern. When the interaction is plotted (Figure 5.4.5b), the two wheat diets appear to have fairly similar arabinose to xylose ratios with age, while the corn diet provided a substantial drop at 21 to 28 d followed by an increase at 35 to 42 d. The low molecular weight fraction of the ileal digesta supernatant interaction (Figure 5.4.5c) shows that while the wheat diet decreases slightly with age, and the enzyme-supplemented diet stays fairly constant, the corn diet results in a substantial increase in arabinose to xylose ratio with age. All of this follows an initial drop from 7 – 14 d in corn and wheat diets. This could not be shown for corn as the amounts of arabinose and xylose in the low molecular weight soluble supernatant were so small that the arabinose to xylose ratio at 7 d yielded positive infinity.

There were no treatment effects on dry matter digestibility or on arabinose, or glucose digestibility (Table 5.4.13). Xylose digestibility was different between the two wheat diets and the corn diet. Arabinose and xylose digestibility values were negligible, however, so any differences seen may have been artifacts. Age did affect digestibility coefficients with dry matter, arabinose, xylose, and glucose all showing

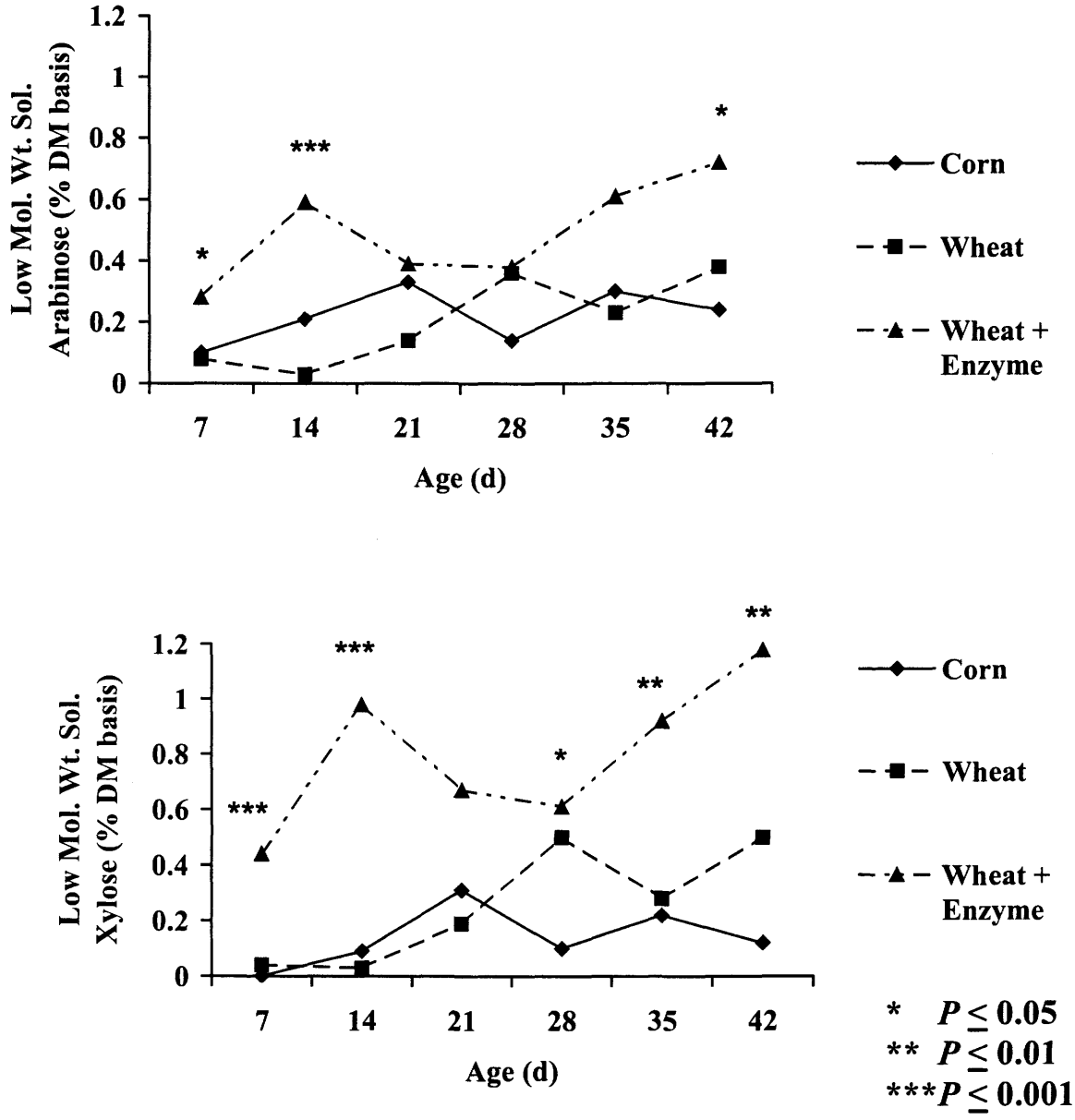

Figure 5.4.4 Effect of diet and age on the residual low-molecular weight (<100 kDa), soluble arabinose and xylose in the terminal ileal digesta of broiler chickens

Table 5.4.12 Effect of diet and age on arabinose to xylose ratio in total, soluble and low molecular weight, soluble terminal ileal fractions from broiler chickens

Variables		Arabinose to Xylose Ratio		
		Whole Ileal Contents	Soluble Ileal Supernatant	Low Molecular Weight, Soluble Ileal Supernatant
Diet	Corn	1.04[a]	1.80[a]	1.60[a]
	Wheat	0.80[b]	0.83[b]	0.78[b]
	Wheat plus Enzyme	0.81[b]	0.76[c]	0.62[c]
Age (d)	7	0.93[a]	1.24[a]	0.50[b]
	14	0.93[a]	1.12[b]	0.96[a]
	21	0.94[a]	1.02[c]	0.92[a]
	28	0.89[b]	1.01[c]	0.96[a]
	35	0.81[c]	1.18[ab]	1.01[a]
	42	0.80[c]	1.20[a]	1.14[a]
Statistics				
Diet		***	***	***
Age		***	***	*
Diet X Age		*	***	*
SEM		0.02	0.06	0.06

[a-c]Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

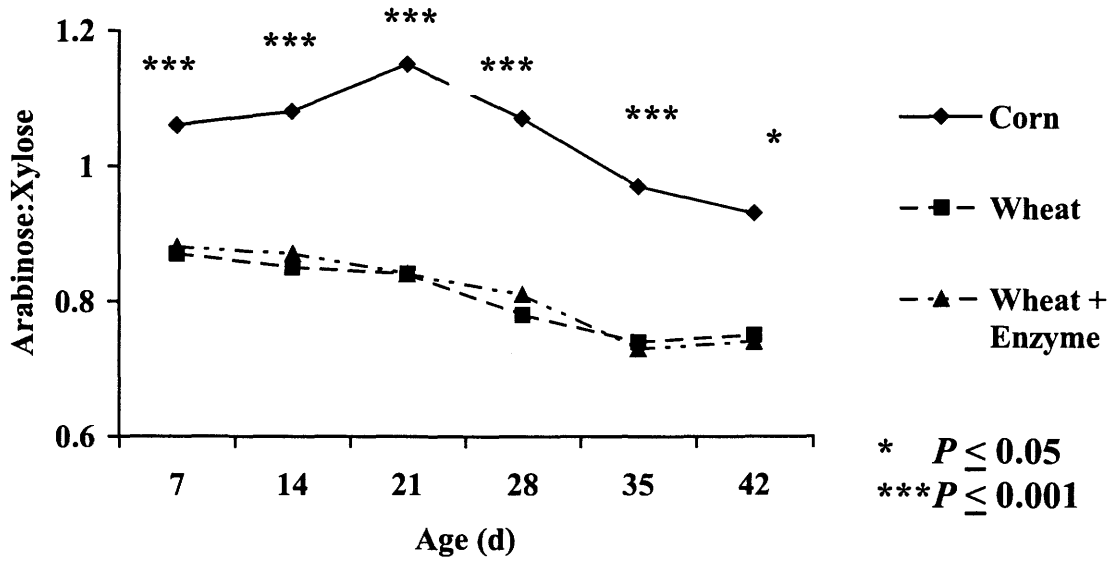

Figure 5.4.5a Effect of diet and age on the arabinose to xylose ratio in whole terminal ileal digesta samples from broiler chickens

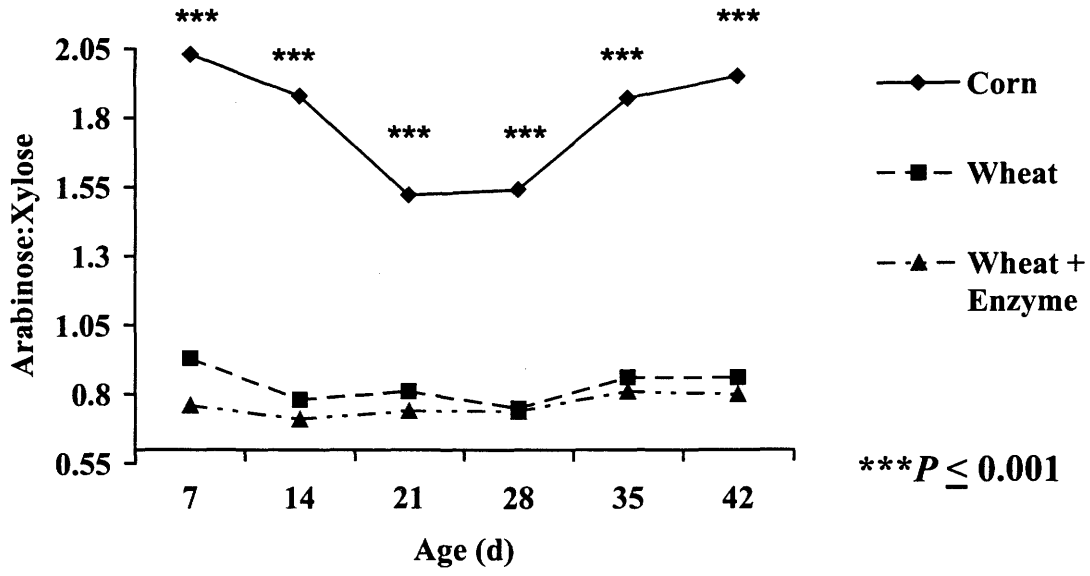

Figure 5.4.5b Effect of diet and age on the arabinose to xylose ratio in the supernatant from terminal ileal digesta samples from broiler chickens

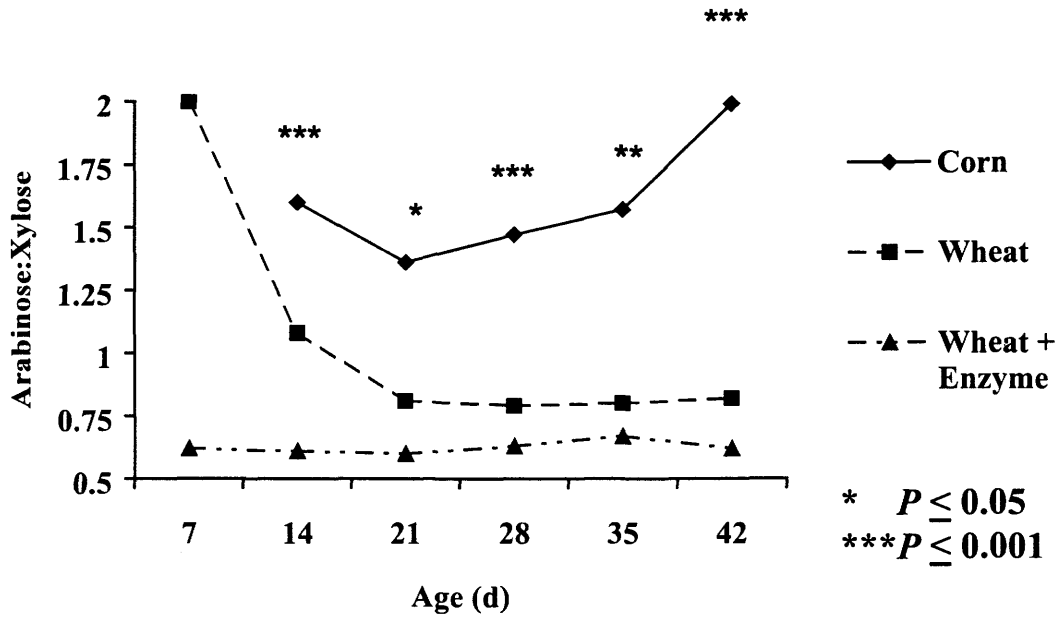

Figure 5.4.5c Effect of diet and age on the arabinose to xylose ratio in a low molecular weight (<100 kDa) fraction of the supernatant from terminal ileal digesta samples from broiler chickens

Table 5.4.13 Effect of diet and age on ileal dry matter digestibility and sugar disappearance

Variables		Nutrients (Ileal Digestibility)			
		Dry Matter	Arabinose	Xylose	Glucose
Diet	Corn	50.76	-6.08	-5.83[a]	40.80
	Wheat	44.91	-6.37	-8.03[b]	35.03
	Wheat plus Enzyme	53.65	-6.63	-8.18[b]	36.55
Age (d)	7	44.24[b]	-6.75[abc]	-7.20[ab]	31.87[c]
	14	47.90[ab]	-7.53[bc]	-8.10[bc]	35.09[bc]
	21	58.23[a]	-4.18[a]	-4.24[a]	42.74[ab]
	28	43.49[b]	-5.73[ab]	-6.62[ab]	28.95[c]
	35	46.45[ab]	-5.42[ab]	-6.94[ab]	44.47[a]
	42	58.34[a]	-8.56[c]	-10.98[c]	41.61[ab]
Statistics					
Diet		NS	NS	0.06	NS
Age		*	*	**	**
Diet X Age		NS	NS	NS	0.11
SEM		1.79	0.38	0.49	1.41

[a-c]Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

an increase in digestibility at 21 d of age. This was followed, in the case of arabinose and xylose, by further decreases, but in the case of dry matter and glucose, by a drop at 28 d, then increases after 35 d.

5.5 Discussion and Conclusions

Performance in the current experiment was excellent across treatments and no enzyme effect on performance was observed. The production response to enzyme supplementation is commonly low when the control or unsupplemented birds have good performance (Willingham *et al.*, 1960; Scott *et al.*, 1998c). The wheat used in the starter and grower diets was replaced by blended feed grade wheat for the finisher phase. Different varieties of wheat have been shown to contain different levels of arabinoxylans and to produce different types of branched oligosaccharides after enzymatic hydrolysis with endo-xylanases (Austin *et al.*, 1999). The structure and relative quantities of the latter oligosaccharides play a significant role in determining the original branched structure of the arabinoxylan and, hence, their viscosity generating properties (Austin *et al.*, 1999). This simple difference in wheat variety may have affected the late cycle data in this experiment. However, the overall *in vitro* viscosity was similar for both the grower and finisher diets so it is unlikely that the change in wheat sample was a major factor. In addition, total sugar analysis of the diets indicated slightly higher levels of arabinose and xylose in the finisher diets, relative to the grower diets, which is likely due to the relatively greater amount of wheat used in these diets rather than any major difference in NSP quantity or composition.

Despite the lack of performance effect, there was a consistent decrease in viscosity as a result of enzyme supplementation, demonstrating that the enzyme was effective. On all diets, viscosity was consistently higher in the ileum than the jejunum. This is logical since the concentration of arabinoxylans in the GIT will increase as the digesta moves down the tract and more absorption of nutrients takes place. An increase in dry matter will also concentrate arabinoxylans and this also occurs with progression down the GIT. This creates an interesting relationship between water content and digesta viscosity.

Dietary effects on viscosity were as expected. Corn diets resulted in consistently low digesta viscosities. Wheat diets supplemented with xylanase resulted in viscosities slightly, but rarely significantly, higher than corn. Unsupplemented wheat diets resulted in the highest viscosities, although the enzyme supplemented diets were similar to wheat at 7 and 35 d.

Age has an interesting effect on viscosity in this experiment with the corn diet being largely unaffected by age. The corn and enzyme-supplemented wheat diets show slight increases in digesta viscosity with increasing concentration of grain in the diets, from starter (57.8% corn; 61.7% wheat) through grower (63.9% corn; 72.2% wheat) to finisher (63.2% corn; 73.9% wheat). The unsupplemented wheat diet, however, resulted in a dramatic increase in digesta viscosity with age, peaking at 21 d, dropping, but remaining significantly higher than the other two diets to 28 d before decreasing to levels similar to enzyme supplemented diets at 35 and 42 d. Petersen *et al.* (1999) similarly evaluated digesta viscosity in broilers fed wheat and barley diets continuously for various age intervals from either 15 or 20 d to 35 or 45

d. Their research resulted in similar ileal viscosities for continuous wheat-fed birds of a similar age to those in the current experiment. While they did not collect data from the early ages used in the current experiment, and therefore did not observe the initial increase in digesta viscosity seen at 21 d, their research did show a reduction of viscosity with age for continuous wheat and barley-fed birds, similar to that seen in the current experiment. They speculated that this decrease in viscosity was likely an adaptation of the microflora in either composition or number, to the presence of the NSP in the diets and a resultant increase in the solubilization of the NSP. The NSP data from the current experiment also support this supposition in that the unsupplemented wheat diet was associated with increased solubilization of both arabinose and xylose with increasing age of the bird. The initial increase in viscosity at 21 d in wheat-fed birds could be due to microbial adaptation resulting in increased solubilization of arabinoxylans but not to very low molecular weight, absorbable compounds. Therefore, as the birds age, either more microbial enzyme is produced, resulting in hydrolysis exceeding release, or, alternatively, the microbes could be adapting and producing different enzymes with different activities, giving the same overall effect.

The small intestine generally increased in size, relative to body weight, with consumption of wheat diets versus corn. In the case of full SI weights and ileal content weights, the unsupplemented wheat diet resulted in more NSP in the GIT and, therefore, heavier weights. Overall, SI measures, both full and empty, are higher for wheat diets likely due to the presence of larger molecular weight and differently structured polysaccharides than those in the corn diet (Austin *et al.*, 1999;

Huisman *et al.*, 2000). There was no dietary effect on dry matter digestibility in the current study, so the GIT size difference between treatments was not an effect of lower digestibility diets leading to larger GIT size. The simple presence of NSP in the GIT has been shown to result in such morphological changes as increased weight and length of SI segments (Johnson and Gee, 1986; Jørgensen *et al.*, 1996). This could be a result of physiological adaptations to increase nutrient absorption either due to the viscous environment (Johnson and Gee, 1981; Johnson and Gee, 1986; Choct and Annison, 1992a) or to competition between the GI bacteria and the bird itself. In germ-free (GF) chickens, Langhout *et al.* (2000) found that added NSP (in this case, highly methylated citrus pectin) increased caecal weight, including contents, over corn diets with no added NSP, but did not have an effect on the GIT itself, as no bacteria were present to ferment them. The presence of bacteria in the conventional birds in this study increased digesta viscosity over that of the GF birds leading to speculation that a bacterial end product may be involved in the increase in GIT size seen with bacterial fermentation. Fecal digestibility of dry matter, organic matter, crude fat and starch, as well as N retention, were all reduced by NSP addition in conventional birds, but were unaffected by NSP addition in GF birds. ME of conventional birds was reduced by the presence of added NSP whereas in GF birds, ME of diets with added NSP was increased. Langhout *et al.* (2000) speculate that changes in the metabolic activity of the GIT microflora, induced by dietary NSP, such as the production of amines, ammonia and other toxins, can have an impact on mucosal morphology and, therefore, nutrient absorption. A difference in small intestinal villus morphology was noted by Langhout *et al.* (2000) with added NSP

reducing numbers of zigzag patterns and ridge-shaped villi and increasing the number of tongue-shaped villi. The actual weights of all GIT components, including contents, were higher in conventional versus GF birds.

In the current study, caecal weights, both full and empty, and caecal lengths are greater from enzyme supplemented, wheat-fed birds than unsupplemented wheat-fed birds. It is of interest that while caecal contents, relative to body weight, are not affected by diet, they do increase with age. This is supportive of the notion that the bacteria of the hindgut develop with age and, therefore, caecal fermentation increases with age on all diets. The increased caecal size with enzyme supplementation is likely a result of increased access to the caeca by low-molecular weight, soluble NSP resulting from enzyme degradation. The NSP data corroborate this in that enzyme supplementation resulted in significantly higher proportions of arabinose and xylose from total, soluble and low molecular weight soluble fractions of the digesta at the terminal ileum. When calculations are made to present soluble and low molecular weight arabinose and xylose as a percentage of total ileal arabinose and xylose, the enzyme-supplemented diets still have significantly higher levels than either of the other diets allowing for increased caecal access by these substrates. In some research there is a significant increase in caecal VFA levels with enzyme supplementation as a result of bacterial fermentation of these substrates (Choct *et al.*, 1995, 1996, 1999). In Choct and coworkers' research, caecal fermentation was increased by enzyme supplementation and was presented on the basis of total VFA content of the caecal digesta. The VFA data from the current experiment does not demonstrate any measurable increase in caecal fermentation with enzyme supplementation of the

wheat diets. Analyzing the current experiment on the same basis as Choct and coworkers' experiments (Table 5.4.14) does not change the fact that, in this case, enzyme did not increase overall caecal fermentation.

In the current experiment VFA levels in the small intestine were not affected by enzyme addition. The viscosity differences in the current experiment, while significant, were not enough to induce measurable decreases in bacterial fermentation in the ileum, with enzyme addition, as was seen by Vahjen *et al.* (1998) and by Choct *et al.* (1996, 1999) in birds aged 7-24 d. Earlier work reported in this thesis (Chapter 3) demonstrated that enzyme supplementation increased acetic, propionic and total VFAs in the ileum, particularly at later ages (42 d). The research of Vahjen *et al.* (1998) demonstrated decreases in some ileal VFA levels with enzyme addition at early ages. Vahjen *et al.* (1998) also noted that unsupplemented wheat-fed birds produced more lactic acid in the ileum than xylanase-supplemented birds. At the same time, xylanase-supplemented birds produced more butyric acid than unsupplemented controls. An important observation from this published research is the gradual increase with age in lactic acid production in the ileum of xylanase-supplemented birds, which, as indicated by Hume *et al.* (1995), can provide caecal bacteria with an intermediate substrate for propionic acid production. Langhout *et al.* (2000) also found elevated lactic acid levels in the chicken ileum with NSP supplementation, while no other differences in VFA levels were observed. Therefore, despite no major VFA differences being observed with enzyme supplementation, there could still have been changes occurring. Lactic acid levels, however, were not measured in the current experiment.

Table 5.4.14 Effect of diet and age on total volatile fatty acid levels (µmol) in the caeca of broiler chickens

Variables		Caeca						
		Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFA
Diet	Corn	258.79	22.91^{a}	2.25^{a}	90.98	3.70	6.32^{a}	384.96
	Wheat	288.41	15.13^{b}	1.75^{ab}	91.04	2.84	4.90^{b}	404.07
	Wheat plus Enzyme	292.21	12.19^{b}	1.49^{b}	101.16	2.85	4.61^{b}	414.51
Age (d)	7	52.69^{d}	2.00^{c}	0.53^{b}	15.24^{c}	0.85^{c}	0.09^{d}	71.39^{d}
	14	74.47^{d}	3.36^{c}	0.41^{b}	17.49^{c}	1.00^{c}	0.67^{d}	97.40^{d}
	21	196.44^{c}	11.47^{b}	1.00^{b}	59.63^{b}	1.97^{c}	2.75^{c}	273.25^{c}
	28	400.14^{b}	27.34^{a}	2.71^{a}	155.25^{a}	4.13^{b}	7.77^{b}	597.34^{b}
	35	435.17^{b}	25.62^{a}	2.76^{a}	151.93^{a}	4.37^{b}	9.27^{b}	629.12^{ab}
	42	519.90^{a}	30.67^{a}	3.57^{a}	166.83^{a}	6.47^{a}	11.12^{a}	738.57^{a}
Statistics								
	Diet	NS	***	*	NS	0.07	**	NS
	Age	***	***	***	***	***	***	***
	Diet X Age	NS	**	NS	NS	NS	0.07	NS
	SEM	24.14	1.75	0.19	8.71	0.29	0.57	34.91

$^{a-e}$Means within a column, within a main effect, with differing superscripts are significantly different (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; NS = not significant).

SEM = pooled standard error of the mean.

The caecal VFA data provide evidence of differential fermentation of residual substrates. In the wheat diets, there was no dramatic effect of enzyme addition on VFA production. In the case of propionic acid production, enzyme supplementation seemed to have no effect whereas propionic acid levels increased to 28 d on the unsupplemented wheat diet. This is in agreement with Choct *et al.* (1999), who did not notice a difference in the molar proportion of propionic acid, or any other VFAs, with enzyme supplementation. The increase noted with age for this treatment might be indicative of the adaptation of the resident flora to the substrate by increasing the proportion of propionic acid producers, as proposed by Apajalahti and Bedford (1998). This concept is also supported by previous work in this thesis (Chapter 3) where higher caecal propionic acid levels were seen with unsupplemented than xylanase-supplemented diets. The propionic acid levels seen in the current study are, however, contradictory to the theory of Apajalahti and Morgan, as explained by Bedford (1996) that propionic acid results from the fermentation of small molecular weight oligosaccharides released from NSP that would not normally have been available to the caecal microbes in the absence of the enzyme. Further work by Apajalahti and Bedford (1998) actually demonstrated an increase in the proportion of propionic acid produced in the caeca of xylanase-supplemented, wheat-fed birds over unsupplemented birds. The reasons for this difference are not clear but probably relate to substrate availability, wheat arabinoxylan composition and GIT conditions such as pH and viscosity. The work of Apajalahti and Bedford (2000) does suggest that wheat diets, in general, tend to favour the growth of propionic acid bacteria.

For the most part, research indicates that the addition of viscosity generating dietary NSP causes increases in butyric acid production, usually at the expense of acetic acid, in the caecum of rats (Key and Mathers, 1993a), rabbits (Jehl and Gidenne, 1996; Gidenne *et al.*, 1998), and in the ileum of wheat-fed chickens (Vahjen *et al.*, 1998). The addition of xylanases to wheat based, viscosity generating poultry diets tended to lower ileal VFA levels (Choct *et al.*, 1995, 1996, 1999), shifting production to the caecum, which is logical since the low molecular weight, soluble NSP substrates prevalent in the ileum of enzyme-treated birds can reach the caeca unimpeded by viscous digesta. This concept is supported by the NSP data of the current experiment.

Of interest is the significant increase in propionic acid production in the caeca of corn-fed birds, particularly from 21 d of age. Higher levels of propionic acid in the caeca of corn-fed birds were also seen in Experiment 1 of Chapter 3 of this thesis where VFA were only measured at 42 d. Propionic acid can be synthesized from a number of sources. Research has documented the fact that increased concentrate feeding leads to an increase in propionic acid production by the rumen bacteria as well as by the bacteria of the hindgut of non-ruminants (van Soest, 1982). This propionic acid results mainly from bacterial fermentation of starch as well as protein, amino acids, and to some degree, fibre. Arabinose from the fibre in cooked haricot beans has been shown to be correlated well with caecal propionic acid production in rats (Key and Mathers, 1993b). In addition, rats fed 100 g/kg of arabinoxylans isolated from corn displayed significant caecal hypertrophy, as well as an accumulation of VFA, particularly propionic acid (Lopez *et al.*, 1999). The end-products of fermentation of any given carbohydrate

substrate depend largely on the microbial species present which is a function of environment as well as the adaptation to the dietary ingredients being fed. Differences in fermentation between the corn and wheat-based diets in the current experiment may be a result of the differences in the composition of the bacterial community as well as the residual substrates available to these bacteria. Apajalahti and Bedford (2000) have demonstrated, using % G + C DNA base composition analysis, that the major groups of bacteria present in the caeca differed between corn, wheat and rye-based diets. It is possible that the GI bacteria present in the corn-fed birds have a higher proportion of propionic acid producers, although these researchers have suggested that wheat diets stimulate the growth of these bacteria. Propionic acid is also produced by anaerobic bacteria from lactic acid provided to them indirectly from carbohydrate fermentation by facultatively anaerobic bacteria (Hume *et al.*, 1995). It is possible that the corn diet stimulates growth of these bacteria.

Alternatively, the high level of propionic acid could be from a different substrate. The ileal digestibility of the starch in corn has been shown to be as low as 85% (Noy and Sklan, 1995). This leads to speculation that the high level of caecal propionic acid in the corn-fed birds, relative to the wheat-fed birds, might be a result of bacterial fermentation of residual starch, itself released by bacterial degradation of the insoluble arabinoxylans in the cell walls of corn. Ruminant research in this area has repeatedly shown higher levels of propionic acid in the rumen with increasing levels of starch from grains (Van Soest, 1982; Pylot *et al.*, 2000). The significantly higher levels of isobutyric and isovaleric acids are possible indicators of bacterial fermentation of

residual protein encapsulating starch in the corn-fed birds (Cummings and Macfarlane, 1991). Huisman *et al.* (2000) found that the composition of water-unextractable solids isolated from corn kernels contained 7% protein and 8% starch, in addition to 57% NSP of a highly substituted, endo-xylanase resistant nature. This concept is supported by the current research where no increase in arabinose or xylose solubility occurred with age in corn-fed birds.

The NSP data from the current experiment indicate an adaptation of the ileal bacteria to the NSP substrate in the wheat diets and a resultant increase in their capacity for fibre degradation. The constant level of soluble arabinose and xylose present in the ileum of corn-fed birds and the fairly steady, higher level of soluble arabinose and xylose in the digesta of enzyme-supplemented birds indicate little adaptation to fibre degradation of the bacteria on these diets. The higher levels of residual NSP in the enzyme-supplemented birds would appear to be indicative of higher nutrient digestibility of this diet (resulting in more of the undigestible material being left behind). Although others have shown this to be true, the dry matter digestibility in the current experiment was only numerically higher for the enzyme-supplemented diet. The bacteria in the GIT of birds on the enzyme diet likely do adapt to the substrates provided but in different ways not detected by the methodology of the current experiment. The steady increase in solubilization of the arabinose and xylose in the terminal ileal contents of the wheat-fed birds, however, provide evidence of bacterial adaptation. Total arabinose and xylose levels remain relatively constant but soluble arabinose and xylose levels increase with age of the bird. This has not been demonstrated prior to this

experiment.

The analysis of the low molecular weight (≤100 kDa) fraction of the ileal digesta supernatant yielded explainable main effects. This fraction was highest from birds fed the enzyme-supplemented wheat diet. Similarly, others have shown that xylanase supplementation of wheat diets increases the amount of a number of smaller molecular weight fractions of NSP over unsupplemented wheat diets (Bedford and Classen, 1992; Apajalahti and Bedford, 1998; Bedford and Apajalahti, 2001). The increase in arabinose and xylose in this fraction with age, although not previously demonstrated, is logical since the bacteria of the GIT are thought to increase with age and the overall capacity of the GIT gets larger with age allowing for greater bacterial fermentation and breakdown of large NSP into lower molecular weight polymers. This data has not clearly shown whether there is an actual increase in total bacterial numbers with age or whether there is simply a change in the balance of bacterial groups present in response to the dietary substrates provided. One could argue that the increases in smaller molecular weight arabinose and xylose with age could also be due to microbial adaptation to increased hydrolysis of soluble NSP to the smaller molecular weight fraction, or to improved enzyme efficacy, or even simply to an increase in the soluble fraction providing more substrate for bacterial hydrolysis to the smaller molecular weight fraction.

The interactions between diet and age are more complex. The amount of arabinose and xylose in the low molecular weight fractions of ileal supernatant from birds fed the enzyme supplemented diet is different from that of the remaining diets

because of the ongoing enzymatic hydrolysis of the arabinoxylans. This is entirely expected as a result of enzyme activity. It is likely that the bacteria develop in response to substrate (Apajalahti and Bedford, 1998) and, over time, are able to hydrolyze some of the NSP present. It is of interest that on each diet, the birds reach a point where either absorption or degradation of smaller NSP catches up to hydrolysis of larger NSP and the increase in low molecular weight NSP levels off. This happens at a different age for each diet. In enzyme-supplemented birds, this happens at 14 d, in corn-fed birds at 21 d, and in wheat-fed birds at 28 d. Since the substrate structure in each case would be different due to differences in arabinoxylan structure and enzymatic degradation (Austin *et al.*, 1999; Huisman *et al.*, 2000) it is likely that the rate at which the GIT bacteria hydrolyze the NSP in each diet would be different. For the corn diet, despite a major increase in total arabinoxylans, the structure of the arabinoxylan is very resistant to hydrolysis (Huisman *et al.*, 2000). As a result there is little increase in either soluble NSP or low molecular weight, soluble NSP with age. For both unsupplemented and enzyme supplemented wheat diets, there are similar levels of total arabinoxylans at all ages. With unsupplemented wheat diets, soluble NSP increases with microbial adaptation, as discussed previously, but there is only a minor increase in the low molecular weight fraction of arabinoxylans. This suggests that the bacteria hydrolyze cell wall material to make it more soluble. Initially the rate of solubilization is higher than the rate of hydrolysis to smaller molecular weight fractions so viscosity increases. Later on, the bacteria reduce digesta viscosity by breaking the cell wall material down more, but these bacteria still don't have the ability to hydrolyze as much material into

the low molecular weight fraction (< 100 kDa) as the exogenous enzyme does (Figure 5.4.4).

This difference in hydrolysis is evident when a comparison is made of the arabinose to xylose ratio (A:X) in the different ileal digesta fractions for the corn diet as compared with the two wheat diets. The major difference seen between corn and the wheat diets is due largely to the differences in NSP structure between the two grains. The corn diet is also higher in soybean meal, which has a higher level of arabinose, but since the level of soybean meal increases in the diets with age and the arabinose to xylose ratio in ileal digesta does not increase on the corn diet with age, it is not likely that the soybean meal is a major factor in the difference between corn and wheat diets. For the wheat diets, the arabinose to xylose ratio in ileal digesta is almost identical, as expected. Very little difference is seen in this ratio for the soluble fraction of the digesta, indicating that a certain portion of the arabinoxylan present in wheat is soluble without enzyme and this fraction would be hydrolyzed regardless of enzyme supplementation. The only notable difference in arabinose to xylose ratio is seen with the low molecular weight fraction where enzyme supplementation appears to result in smaller molecular weight arabinoxylans with fewer arabinose substitutions on them. This could be due to the enzyme having side activities which could cleave arabinose residues off the arabinoxylans leaving better access to xylose-xylose bonds for the endoxylanase, and, as a result, more small molecular weight arabinoxylans. These low molecular weight compounds could have a prebiotic effect in the GIT and be one of the reasons for enzymes sometimes yielding positive performance effects in older broiler

chickens even when there is no effect of the enzyme on digesta viscosity.

The major finding from the current study involves the change in level of soluble, residual NSP with age of the bird on unsupplemented wheat diets. Since levels of arabinose and xylose in whole ileal samples do not change with age, and are fairly constant with age on both the corn and the enzyme diet, but levels of soluble arabinose and xylose increase with age on the unsupplemented wheat diet, it is likely that the bacterial flora adapt to the presence of high molecular weight NSP and adjust their community composition to be able to solubilize this material. There are no other studies in the literature examining this change in ileal digesta composition with age. The research supporting this hypothesis includes studies which demonstrate that time is required for complete physical adaptation of the GIT to diets high in fibre, anywhere from 1 week to 1 month in rats (Brunsgaard *et al.*, 1995) and from 4-8 weeks in galliforme birds (Redig, 1989). Other research that shows clear distinctions between the bacteria present on unsupplemented versus enzyme-supplemented wheat diets (Vahjen *et al.*, 1998; Dänicke *et al.*, 1999; Apajalahti and Bedford, 1998) and fermentation differences between the two types of diet (Choct *et al.*, 1996, 1999) also support this theory. This adaptation in bacterial flora is likely a partial explanation for improvements seen in performance and digesta viscosity with age of birds fed wheat diets. In addition, it explains some of the reduced efficacy of xylanase supplementation on digesta viscosity in broilers at later ages while still allowing for the existence of a positive, prebiotic effect of the low molecular weight products of enzyme hydrolysis even when viscosity is no longer an issue.

In conclusion, there appears to be an age effect on both viscosity and bacterial solubilization of dietary NSP resulting in different substrates remaining at the terminal ileum of the broiler chicken, relating to the diet fed. Further research would best be directed at more detailed evaluation of the NSP compounds at the terminal ileum and determining which of these enter the caeca. In addition, more exact evaluation of the source of some of the differences in caecal VFA production would be of interest.

6.0 GENERAL DISCUSSION

Some of the implications of dietary NSP and enzyme supplementation on the GIT bacteria of the broiler chicken have been elucidated in the current studies. The effectiveness of enzyme-supplementation has been shown to depend, to some extent, on the performance level of the birds. This is not a new concept as others have indicated that response of birds to enzyme supplementation depends on the amount of room for improvement. Low AME grains or those resulting in poorer bird performance will generally give a much greater response in AME or performance to enzyme supplementation than high AME or higher performance yielding grains (Willingham *et al.*, 1960; Scott *et al.*, 1998c; Rosen, 2001).

While the bacterial and fermentation data from the experiments described in Chapter 3 at first glance appear to contradict much of the literature, by indicating increased ileal fermentation with enzyme and decreased caecal fermentation under the same conditions, this may be a true age-related bacterial adaptation. The discrepancies may be explained by the different ages of the birds used in the current study, compared with those described in the literature. The bacterial data collected and analysed in the current studies were taken from birds that were 28 and 42 d of age whereas much of the published literature discusses bacterial culturing on birds from 7-21 d of age (Choct *et al.*, 1995; Choct *et al.*, 1996; Langhout, 1998; Vahjen *et al.*, 1998; Dänicke *et al.*, 1999). In addition, the digesta viscosities generated by the diets used in the current

studies are lower and more representative of actual poultry diets than many published studies, which has an impact on the extent of bacterial overgrowth in the ileum and the changes that occur with age (Choct *et al.*, 1996; Dänicke *et al.* 1997a,b; 1999; Langhout *et al.*, 1999). In the Langhout *et al.* (1999) study, the treatment with low methylated citrus pectin (LMC, as an added source of fermentable NSP) did not elevate digesta viscosity as much as the high methylated citrus pectin (HMC) and, consequently, did not result in significantly different bacterial levels from the corn-based control diet. Viscosities resulting from the addition of LMC were significantly higher than those of the corn-fed control and were similar to the wheat-fed birds in the current study, as were the viscosities observed by Vahjen *et al.* (1998), who fed diets similar to those used in the current study. Vahjen *et al.* (1998) observed that bacterial proliferation in the ileum was reduced with xylanase supplementation of wheat diets in birds at 7 and 14 d but not significantly by 21 and 28 d of age. The later ages confirm the observations made that endoxylanase supplementation of wheat diets had little effect on bacteria at 28 d of age and thereafter in the current studies.

Traditional culturing methods are useful for determining the effect of dietary changes on specific groups of bacteria, but need to be carried out with great care in a microbiology laboratory to allow for the most accurate results within the limitations of the technique (Hungate,1950; Amann *et al.*, 1995). These techniques also only provide access to a very small proportion of the total bacterial community present, even if carried out perfectly. Ideally, due to some of these limitations, DNA-based analyses would be of use to complement traditional culturing. For bacterial groups, the %G+C

DNA base composition analysis is effective and accurate (Apajalahti and Bedford, 1998; Apajalahti *et al.*, 1998; Bedford and Apajalahti, 2001). If actual sub-species or species differences are important, as they have been shown to be with complex changes occurring in response to changes in dietary substrate (Bedford and Apajalahti, 2000), then more precise techniques using 16S ribosomal RNA and in situ hybridization are required. These techniques have been effective in identifying phylogenetic differences not possible using traditional culturing methods (Amann *et al.*, 1995; Langendijk *et al.*, 1995; Snel *et al.*, 1995).

For the purposes of the current studies, the use of VFA levels in the intestinal contents of the birds as a measure of bacterial activity provided useful information on the effect of dietary NSP, enzyme supplementation and age on the GIT microflora. The bacterial data of the first three experiments (Chapters 3 & 4) combined with the VFA data in the first two experiments (Chapter 3), overall, indicated that, although performance response to enzyme-supplementation was as expected, bacteria at 42 d proliferated in the caecum without enzyme and in the ileum with enzyme supplementation. At 28 d the findings were more similar to literature findings (Choct *et al.*, 1996; Vahjen *et al.*, 1998; Choct *et al.*, 1999) with ileal bacterial activity being higher without enzyme. The change between 28 and 42 d is due to a suspected development of the GIT flora with age and adaptation to the dietary NSP substrate provided by the ration. Bedford and Classen (1992) observed that both the molecular weight distribution of residual NSP and the actual sugar composition of the NSP in the broiler GIT changes with differing levels of dietary rye substitution for wheat and

differing levels of enzyme supplementation. Vahjen *et al.* (1998) showed that xylanase-supplementation of wheat-based diets resulted in lower levels of enterobacteria and gram-positive cocci but higher levels of tissue-associated *Lactobacillus* spp. in broilers up to 21d. These researchers also noted a difference in the types and quantities of fermentation end-products in the GIT, indicative of differences in substrate availability. The differences in the composition of the GIT bacterial communities in broiler chickens fed wheat, rye and corn-based diets seen by Apajalahti and Bedford (2000) were said to be due to the differences in substrate available to the bacteria for fermentation. Some of the ileal microflora are suspected of adapting to and preferentially utilizing depolymerized xylo-oligomers generated by enzyme activity (Apajalahti and Bedford, 1998). It is logical to speculate that the 42 d flora, through adaptation to available substrate, may be different enough from the immature 28 d flora, that it can better handle the larger xylo-oligomers in the unsupplemented wheat diets.

In addition, the GIT data (Chapters 3 and 5) reveals that, while no differences in GIT size exist between enzyme-supplemented and unsupplemented wheat diets, there is substantial development with age. Therefore, the mature ileal flora may also, simply as a result of greater fermentative capacity, be capable of generating more VFAs. Again, there are no available published results examining the effect of dietary NSP and enzyme on the GIT bacteria between ages 28 and 42 d.

The results of the final experiment (Chapter 5) clearly demonstrate bacterial adaptation to be occurring. This is evident because the residual NSP substrates present in the terminal ileum are significantly affected by dietary treatment and, in the case of

the unsupplemented, wheat-fed birds, change with age, indicating both dietary differences in substrate and adaptation of the bacteria to the available substrate over time. This is logical since Barnes *et al.* (1972) determined that it takes about 6 weeks to establish an adult caecal flora in chickens. Duke *et al.* (1984) found that turkeys preconditioned to high fibre diets appeared to adapt and to be able to digest fibre by 15 weeks of age. Redig (1989) suggests that poultry require from 4-8 weeks to adapt to a high fibre diet. The current studies all provided evidence of microfloral adaptation to diet between 4 and 6 weeks of age. Since little has been published in recent years on bacterial development in the GIT of poultry (Mead, 2000), this observation merits further study.

Further evidence of the substrate differences can be seen from the differences in fermentation end-products in the caecum. The bacteria of the hindgut produced much higher levels of propionic acid on the corn diet than on either wheat diet. This demonstrates the difference in the amount and type of soluble substrate entering the caeca in birds on different diets. Corn has a somewhat lower amount of NSP but the NSP present is largely insoluble (Shelton and Lee, 2000) and is highly resistant to hydrolysis. Due to the relatively high digestibility of nutrients in the corn diet and the rapid rate of passage of digesta in birds, any starch bound in this resistant NSP would not be released until it was hydrolysed by the more stable caecal bacteria of the bird. This would result in the production of propionic acid either directly from starch fermentation or indirectly via lactic acid production (Hume *et al.*, 1995). Apajalahti and Bedford (1998) demonstrated differences in caecal bacterial community composition

between birds fed corn and wheat. They suggested these differences might be due to differences in substrate availability between the diets. This is supported by the differences seen in arabinose and xylose levels in the ileum of the corn versus the wheat-fed birds.

The performance improvements seen both with enzyme supplementation of wheat diets and with corn-based versus unsupplemented wheat diets (Chapters 3 & 4) provide further evidence for the substrate scenario. Bacteria were shown to be different on the diets, despite equivalent performance, indicating substrate availability differences. Langhout (1998) concludes that differences in the quality of feedstuffs high in NSPs may be related to the structure and composition of the water-soluble NSP fractions that would affect the fermentability of the NSPs. Austin *et al.* (1999) provide concrete evidence for this difference in fermentability in the degree of branching of the arabinoxylans and the relative amount of a xylanase-resistant fraction of NSP. Langhout *et al.* (1999) also demonstrate that the negative effect of water soluble NSP on broiler performance and nutrient digestibility is largely due to the affect this fraction has on the GIT microflora.

The current research has, therefore, demonstrated that the GIT microflora of birds fed diets of differing NSP contents (corn versus wheat), adapt to the type and to the size of NSP present. Enzyme supplementation affects this adaptation. Birds fed unsupplemented wheat diets develop a flora that, around 28d of age, becomes able to solubilize insoluble arabinoxylans, as well as degrade the soluble arabinoxylans to lower molecular weight polymers. Birds fed corn diets appear to be unable to degrade

all of the carbohydrate, including starch, in their diet which may be bound in the highly resistant arabinoxylans of the corn kernel, and which, therefore are degraded by the microflora of the caeca, releasing propionic acid. All of this information points to the possibility of providing exogenous enzymes in the diet of birds which can either hydrolyze NSP high enough in the GIT to provide absorbable substrates to the bird in the upper tract or, further down the GIT to provide substrates of benefit to the desirable microbial population of the caecum, encouraging gut health and discouraging colonization by pathogens.

7.0 REFERENCES

Almirall, M. and E. Esteve-Garcia. 1994. Rate of passage of barley diets with chromium oxide: Influence of age and poultry strain and effect of ß-glucanase supplementation. Poultry Sci. 73: 1433-1440.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59:143-169.

Amat, C., J.M. Planas and M. Moreto. 1996. Kinetics of hexose uptake by the small and large intestine of the chicken. Am. J. Physiol. 271:R1085-R1089.

Angkanaporn, K., M. Choct, W.L. Bryden, E.F. Annison and G. Annison. 1994. Effects of wheat pentosans on endogenous amino acid losses in chickens. J. Sci. Food Agric. 66:399-404.

Annison, E.F., K.J. Hill, and R. Kenworthy, 1968. Volatile fatty acids in the digestive tract of the fowl. Br. J. Nutr. 22:207-216.

Annison, G. 1990. Polysaccharide composition of Australian wheats and the digestibility of their starches in broiler chicken diets. Aust. J. Expt. Agric. 30:183-186.

Annison, G. 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. J. Agric. Food Chem. 39:1252-1256.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. Aust. J. Agric. Res. 44:405-422.

Annison, G. and M. Choct. 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World's Poult. Sci. J. 47:232-242.

Annison, G., M. Choct, and R.J. Hughes. 1995. Enzymes and the nutritive value of lupins. Proc. Aust. Poult. Sci. Sym. 1995:126-129.

Annison, G., R.J. Hughes and M. Choct. 1996. Effects of enzyme supplementation on the nutritive value of dehulled lupins. Br. Poult. Sci. 37:157-172.

Annison, G., M. Choct and N.W. Cheetham. 1992. Analysis of wheat arabinoxylans from a large-scale isolation. Carbohydr. Polym. 19:151-159.

Antoniou, T., R.R. Marquardt, and R. Misir. 1980. The utilization of rye by growing chicks as influenced by calcium, vitamin D-3, and fat type and level. Poultry Sci. 59:758-769.

Antoniou, T., R.R. Marquardt, and E. Cansfield. 1981. Isolation, partial characterization and anti nutritional activity of a factor (pentosans) in rye grain. J. Agric. Food Chem. 29:1240-1247.

Antoniou, T. and R.R. Marquardt. 1982. Utilization of rye diets by chicks as affected by lipid type and level and penicillin supplementation. Poultry Sci. 61:107-116.

Apajalahti, J. and M.R. Bedford. 1998. Nutrition effects on the microflora of the GI tract. Proc. 19th Western Nutrition Conference, Saskatoon, SK, Canada. 1998:60-68.

Apajalahti, J. and M.R. Bedford. 2000. Impact of dietary and environmental factors on microbial communities of the avian GI tract. Proc. XXI World's Poult. Cong. 2000: CD-ROM.

Apajalahti, J., L.K. Sarkilahti, B.R.E. Maki, P. Heikkinen, P. Nurminen and W.E. Holben. 1998. Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. Appl. Environ. Microbiol. 64:4084-4088.

Austin, S.C., J. Wiseman and A. Chesson. 1999. Influence of non-starch polysaccharide structure on the metabolisable energy of U.K. wheat fed to poultry. J. Cereal Sci. 29:77-88.

Bach Knudsen, K.E. and B. Li. 1991. Determination of oligosaccharides in protein-rich feedstuffs by gas-liquid chromatography and high-performance liquid chromatography. J. Agric. Food Chem. 39:689-694.

Bach Knudsen, K.E., B.B. Jensen, and I. Hansen. 1993. Digestion of polysaccharides and other major components in the small and large intestine of pigs fed diets consisting of oat fractions rich in β-D-glucan. Br. J. Nutr. 70:537-556.

Bacic, A. and B.A. Stone. 1981. Chemistry and organization of aleurone cell wall components from wheat and barley. Aust. J. Plant Physiol. 8:475-495.

Bailey, J.S., L.C. Blankenship, and N.A. Cox. 1991. Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. Poultry Sci. 70:2433-2438.

Baker, D. 1977. Xylose and xylan utilization by the chick. Poultry Sci. 56:2105-2107.

Baker, D., K.H. Norris, and B.W. Li, 1979. Food fiber analysis: Advances in methodology. Pages 67-78 *in*: Dietary Fibers: Chemistry and Nutrition. G.E. Inglett and I. Falkehag, ed. Academic Press, London.

Barnes, E.M., G.C. Mead, D.A. Barnum and E.G. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311-326.

Barnes, E.M., C.S. Impey and B.J.H. Stevens. 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hygiene 82:263-283.

Barnes, E.M. and C.S. Impey. 1974. The occurence and properties of uric acid decomposing anaerobic bacteria in the avian caecum. J. Appl. Bacteriol. 37:393-409.

Barrow, P.A. 1992. Probiotics for chickens. Pages 225-257 *in:* Probiotics. The Scientific Basis. R. Fuller, ed. Chapman and Hall, London, UK.

Bedford, M.R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. Anim. Feed Sci. Technol. 53:145-155.

Bedford, M.R. 1996a. Interaction between ingested feed and the digestive system in poultry. J. Appl. Poult. Res. 5:86-95.

Bedford, M.R. 1996b. The effect of enzymes on digestion. J. Appl. Poult. Res. 5:370-378.

Bedford, M.R. and J. Apajalahti. 2001. Microbial interactions in the response to exogenous enzyme utilization. Pages 299-314 *in:* Enzymes in Farm Animal Nutrition, M.R. Bedford and G.G. Partridge eds., CAB International, Oxon, UK.

Bedford, M.R. and K. Autio. 1996. Microscopic examination of feed and digesta form wheat-fed broiler chickens and its relation to bird performance. Poultry Sci. 75(Supp):14 (Abs#53).

Bedford, M.R. and H.L. Classen. 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122:560-569.

Bedford, M.R. and H.L. Classen. 1993. An *in vitro* assay for prediction of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. Poultry Sci. 72:137-143.

Bedford, M.R. and A.J. Morgan. 1996. The use of enzymes in poultry diets. World's Poult. Sci. J. 52:61-68.

Bedford, M.R. and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11:91-114.

Bedford, M.R., H.L. Classen, and G.L. Campbell. 1991. The effect of pelleting, salt and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. Poultry Sci. 70:1571-1577.

Bjornhag, G., 1989. Transport of water and food particles through the avian ceca and colon. J. Exp. Zool. Suppl.3:32-37.

Bogner, P.H. 1961. Alimentary absorption of reducing sugars by embryos and young chicks. Proc. Soc. Exp. Biol. Med. 107:263-267.

Bolton, W., and W.A. Dewar, 1964. The digestibility of acetic, propionic and butyric acids by the fowl. Br. Poult. Sci. 6:103-105.

Boyd, F.M. and H.M. Edwards Jr. 1967. Fat absorption by germ-free chicks. Poultry Sci. 46:1481-1483.

Braun, E.J., and G.E. Duke, 1989. Function of the Avian Cecum: First International Avian Cecal Symposium. J. Exp. Zool. Suppl.3:1.

Braun, E.J., and C.E. Campbell, 1989. Uric acid decomposition in the lower gastrointestinal tract. J. Exp. Zool. Suppl.3:70-74.

Brenes, A., J. Treviño, C. Centeno and P. Yuste. 1989. The influence of alpha-galactosides extracted from lupin seed (*Lupinus albus*) on the digestion of dietary starch by growing chicks. Pages 374-377 *in:* Recent Advances of Research in Antinutritional Factors in Legume Seeds. Proceedings of the First International Workshop on Antinutritional Factors in Legume Seeds, Wageningen, Nethelands, November 23-25, 1988. J. Huisman, T.F.B. Van der Pool and I.E. Leiner, eds. Pudoc, Wageningen.

Brenes, A., R.R. Marquardt, W. Guenter and B.A. Rotter. 1993. Effect of enzyme supplementation on the nutritional value of raw, autoclaved, and dehulled lupins (*Lupinus albus*) in chicken diets. Poultry Sci. 72:2281-2293.

Brown, I. 1996. Complex carbohydrates and resistant starch. Nutr. Rev. 54:S115-S119.

Brunsgaard, G., K.E. Bach Knudsen, and B.O. Eggum. 1995. The influence of the period of adaptation on the digestibility of diets containing different types of indigestible polysaccharides in rats. Br. J. Nutr. 74:833-848.

Bryant, M.P. 1997. Introduction to gastrointestinal microbial ecology. Pages 3-12 *in:* Gastrointestinal Microbiology, Vol. 1. R.I. Mackie and B.A. White, eds. Chapman & Hall, New York.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1 and 2. 1993. Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Campbell, G.L. and M.R. Bedford. 1992. Enzyme applications for monogastric feeds: A review. Can. J. Anim. Sci. 72:449-466.

Campbell, G.L., L.D. Campbell, and H.L. Classen. 1983. Utilisation of rye by chickens: effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. Br. Poult. Sci. 24:191-203.

Campbell, G.L., F.W. Sosulski, H.L. Classen, and G.M. Ballance. 1987. Nutritive value of irradiated and ß-glucanase-treated wild oat groats (*Avena fatua* L.) for broiler chickens. Anim. Feed Sci. Technol. 16:243-252.

Carré, B. and B. Leclercq. 1985. Digestion of polysaccharides, protein and lipids by adult cockerels fed on diets containing a pectic cell wall material from white lupin (*Lupinus albus* L.) cotyledon. Br. J. Nutr. 54:669-680.

Carré, B., B. Prévotel, and B. Leclercq. 1984. Cell wall content as a predictor of metabolisable energy value of poultry feedingstuffs. Br. Poult. Sci. 25:561-572.

Carré, B., L. Derouet, and B. Leclercq. 1990. Digestibility of cell wall polysaccharides from wheat (bran or whole grain), soyabean and white lupin meal in cockerels, Muscovy ducks and rats. Poultry Sci. 69:623-633.

Carré, B., J. Gomez and A. M. Chagneau. 1995. Contribution of oligosaccharide and polysaccharide digestion, and excreta losses of lactic acid and short chain fatty acids, to dietary metabolisable energy values in broiler chickens and adult cockerels. Br. Poult. Sci. 36:611-629.

Castañón, J.I.R., M.P. Flores, and D. Pettersson. 1997. Mode of degradation of non-starch polysaccharides by feed enzyme preparations. Anim. Feed Sci. Technol. 68:361-365.

Chambers, J.R., J.L. Spencer, and H.W. Modler. 1997. The influence of complex carbohydrates on *Salmonella typhimurium* colonization, pH, and density of broiler ceca. Poultry Sci. 76:445-451.

Chaplin, S.B., 1989. Effect of cecectomy on water and nutrient absorption in birds. J. Exp. Zool. Suppl.3:81-86.

Chesson, A. 1987. Supplementary enzymes to improve the utilization of pig and poultry diets. Pages 71-89 *in:* Recent Advances in Animal Nutrition - 1987. W. Haresign and D.J.A. Cole, eds. Butterworths, England.

Chesson, A. 1995. Dietary Fiber. Pages 547-576 *in:* Food Polysaccharides and Their Applications. A. M. Stephen, ed. Marcel Dekker, Inc., New York.

Chesson, A. 2000. Non-starch polysaccharide degrading enzymes-types and methods of action. Proc. XXI World's Poult. Cong. 2000:CD-ROM.

Choct, M. 1999. Soluble non-starch polysaccharides affect net utilisation of energy by chickens. Pages 31-35 *in:* Recent Advances in Animal Nutrition in Australia, Vol. 12., Corbett, J.L. ed., University of New England, Armidale, Australia.

Choct, M. 2001. Enzyme supplementation of poultry diets based on viscous cereals. Pages 145-160 *in:* Enzymes in Farm Animal Nutrition, M.R. Bedford and G.G. Partridge eds., CAB International, Oxon, UK.

Choct, M. and G. Annison. 1990. Antinutritive activity of wheat pentosans in broiler diets. Br. Poult. Sci. 31:811-821.

Choct, M. and G. Annison. 1992a. The inhibition of nutrient digestion by wheat pentosans. Br. J. Nutr. 67:123-132.

Choct, M. and G. Annison. 1992b. Anti-nutritive effect of wheat pentosans in broiler chickens: Role of viscosity and gut microflora. Br. Poult. Sci. 33:821-834.

Choct., M., G. Annison, and R.P. Trimble. 1992. Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. J. Nutr. 122:2457-2465.

Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan and G. Annison. 1995. Feed enzymes eliminate the anti-nutritive effect of non-starch polysaccharides and modify fermentation in broilers. Proc. Aust. Poult. Sci. Sym. 1995:121-125.

Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609-621.

Choct, M., R.J. Hughes, and M.R. Bedford. 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. Br. Poult. Sci. 40:419-422.

Classen, H.L., G.L. Campbell, B.G. Rossnagel, R. Bhatty and R.D. Reichert. 1985. Studies on the use of hulless barley in chick diets: Deleterious effects and methods of alleviation. Can. J. Anim. Sci. 65:725-733.

Classen, H.L., G.L. Campbell and J.W.D. Grootwassink. 1988. Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. Can. J. Anim. Sci. 68:1253-1259.

Coates, M.E., C.B. Cole, R. Fuller, S.B. Houghton, and H. Yokota. 1981. The gut microflora and the uptake of glucose from the small intestine of the chick. Br. Poult. Sci. 22:289-294.

Cole, C.B., R. Fuller, and M.E. Coates. 1981. Effect of the gut flora on lipid absorption in the chick. Pages 365-367 *in:* Recent Advances in Germfree Research. S. Sasaki et al., eds. Tokai University Press, Japan.

Corrier, D.E., A. Hinton Jr., R.L. Ziprin, and J.R. DeLoach. 1990a. Effect of dietary lactose on salmonella colonization of market-age broiler chickens. Avian Dis. 34:668-676.

Corrier, D.E., A. Hinton Jr., R.L. Ziprin, R.C. Beier, and J.R. DeLoach, 1990b. Effect of dietary lactose on cecal pH, bacteriostatic volatile fatty acids, and *salmonella typhimurium* colonization of broiler chicks. Avian Dis. 34:617-625.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, A.G. Hollister, and J.R. DeLoach. 1995. Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria. Poultry Sci. 74:916-924.

Cowan, W.D. 1995. The relevance of intestinal viscosity on performance of practical broiler diets. Proc. Aust. Poult. Sci. Sym. 1995:116-120.

Croucher, S.C. and E.M. Barnes. 1983. The occurence and properties of *Gemminger formicilis* and related anaerobic budding bacteria in the avian caecum. J. Appl. Bacteriol. 54:7-22.

Cummings, J.H. and G.T. Macfarlane. 1991. The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. 70:443-459.

Dänicke, S., O. Simon, H. Jeroch, and M. Bedford, 1997a. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. Physico-chemical chyme features. Br. Poult. Sci. 38:537-545.

Dänicke, S., O. Simon, H. Jeroch, and M. Bedford. 1997b. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 2. Performance, nutrient digestibility and the fat-soluble vitamin status of livers. Br. Poult. Sci. 38:546-556.

Dänicke, S., W. Vahjen, O. Simon, and H. Jeroch, 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. Poultry Sci. 78:1292-1299.

Dantzer, V., 1989. Ultrastructural differences between the two major components of chicken ceca. J. Exp. Zool. Suppl.3:21-31.

Duke, G.E. 1993. Avian digestion. Pages 428-436 *in:* Dukes' Physiology of Domestic Animals, 11th Edition. M.J. Swenson and W.O. Reece, eds. Cornell University Press, Ithaca.

Duke, G.E., E. Eccleston, S. Kirkwood, C.F. Louis, and H.P. Bedbury, 1984. Cellulose digestion by domestic turkeys fed low or high fiber diets. J. Nutr. 114:95-102.

Duke, G.E., O.A. Evanson, and D.R. Epstein, 1983. Coordination of cecal motility during cecal evacuation. Poultry Sci. 62:545-550.

Ellis, P.R., P. Rayment and Q. Wang. 1996. A physico-chemical perspective of plant polysaccharides in relation to glucose absorption, insulin secretion and the entero-insular axis. Proc. Nutr. Soc. 55:881-898.

Elwinger, K. and B. Teglöff. 1991. Performance of broiler chickens as influenced by a dietary enzyme complex with and without antibiotic supplementation. Arch. Geflügelk. 55:69-73.

Englyst, H.N., 1989. Classification and measurement of plant polysaccharides. Anim. Feed Sci. Technol. 23:27-42.

Englyst, H.N. and G.J. Hudson. 1987. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food Chem. 24:63-76.

Eyssen, H. and P. De Somer. 1967. Effects of *Streptococcus faecalis* and a filterable agent on growth and nutrient absorption in gnotobiotic chicks. Poultry Sci. 46:323-333.

Feighner, S.D. and M.P. Dashkevicz. 1988. Effect of dietary carbohydrates on bacterial cholyltaurine hydrolase in poultry intestinal homogenates. Appl. Environ. Microbiol. 54:337-342.

Fengler, A.I. and R.R. Marquardt. 1988a. Water-soluble pentosans from rye: II. Effects on rate of dialysis and on the retention of nutrients by the chick. Cereal Chem. 65:298-302.

Fengler, A.I. and R.R. Marquardt. 1988b. Water-soluble pentosans from rye: I. Isolation, partial purification, and characterization. Cereal Chem. 65:291-297.

Fengler, A.I., J.R. Pawlik, and R.R. Marquardt. 1988. Improvement in nutrient retention and changes in excreta viscosities in chicks fed rye-containing diets supplemented with fungal enzymes, sodium taurocholate and penicillin. Can. J. Anim. Sci. 68:483-491.

Ferrer, R., J.M. Planas, and M. Moretó. 1986. Characteristics of the chicken proximal cecum hexose transport system. Pflügers Arch. 407:100-104.

Ferrer, R., J.M. Planas, and M. Moretó. 1995. Cell apical surface area in enterocytes from chicken small and large intestine during development. Poultry Sci. 74:1995-2002.

Ferrer, R., J.M. Planas, M. Durfort, and M. Moreto, 1991. Morphological study of the caecal epithelium of the chicken (*Gallus Gallus Domesticus L.*). Br. Poult. Sci. 32:679-691.

Fincher, G.B. and B.A. Stone. 1986. Cell walls and their components in cereal grain technology. Pages 207-295 *in:* Advances in Cereal Science and Technology, Vol. 8. Y. Pomeranz, ed. AACC, Minnesota.

Fischer, E. N. 1999. Competitive exclusion as a pathogen control tool in poultry. Proc. 20th Western Nutrition Conference, Calgary, AB, Canada. 20:31-47.

Ford, D.J. 1974. The effect of the microflora on gastrointestinal pH in the chick. Br. Poult. Sci. 14:131-140.

Friesen, O.D., W. Guener, B.A. Rotter and R.R. Marquardt. 1991. The effects of enzyme supplementation on the nutritive value of rye grain (*Secale cereale*) for the young broiler chick. Poultry Sci. 70:2501-2508.

Friesen, O.D., W. Guenter, R.R. Marquardt and B.A. Rotter. 1992. The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats and rye for the young broiler chick. Poultry Sci. 71:1710-1721.

Fuller, R. 1977. The importance of lactobacilli in maintaining normal microbial balance in the crop. Br. Poult. Sci. 18:85-94.

Fuller, R. 1989. A review. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Fuller, R., M.E. Coates, and G.F. Harrison. 1979. The influence of specific bacteria and a filterable agent on the growth of gnotobiotic chicks. J. Appl. Bacteriol. 46:335-342.

Gasaway, W.C., 1976a. Seasonal variation in diet, volatile fatty acid production and size of the cecum of rock ptarmigan. Comp. Biochem. Physiol. [A] 53:109-114.

Gasaway, W.C., 1976b. Volatile fatty acids and metabolizable energy derived from cecal fermentation in the willow ptarmigan. Comp. Biochem. Physiol. [A] 53:115-121.

Gasaway, W.C., 1976c. Cellulose digestion and metabolism by captive rock ptarmigan. Comp. Biochem. Physiol. [A] 54:179-182.

Gasaway, W.C., D.F. Holleman, and R.G. White. 1975. Flow of digesta in the intestine and cecum of the rock ptarmigan. Condor 77:467-474.

Gidenne, T., R. Bellier, and J. van Eys. 1998. Effect of the dietary fibre origin on the digestion and on the caecal fermentation pattern of the growing rabbit. Anim. Sci. 66:509-517.

Gitzelmann, R. and S. Auricchio. 1965. The handling of soya alpha-galactosides by a normal and a galactosemic child. Pediatrics 36:231-235.

Goldstein, D.L., 1989. Absorption by the cecum of wild birds: Is there interspecific variation? J. Exp. Zool. Suppl.3:103-110.

Graham, H. and D. Balnave. 1995. Dietary enzymes for increasing energy availability. Pages 295-309 *in:* Biotechnology in Animal Feeds and Animal Feeding. R.J. Wallace and A. Chesson, eds. VCH, Weinheim, Germany.

Grootwassink, J.W.D., G.L. Campbell, and H.L. Classen. 1989. Fractionation of crude pentosanase (arabinoxylanase) for improvement of the nutritional value of rye diets for broiler chickens. J. Sci. Food Agric. 46:289-300.

Grubb, B.R., 1991. Avian cecum: role of glucose and volatile fatty acids in transepithelial ion transport. Am. J. Physiol. 260:G703-G710.

Haberer, B., E. Schulz, K. Aurlich, and G. Flachowsky. 1998. Effects of ß-glucanase and xylanase supplementation in pigs fed a diet rich in nonstarch polysaccharides: composition of digesta in different prececal segments and postprandial time. J. Anim. Physiol. Anim. Nutr. 78:84-94.

Harrison, G.F. and M.E. Coates. 1972. Interrelationship between the growth-promoting effect of fish solubles and the gut flora of the chick. Br. J. Nutr. 28:213-221.

Havenaar, R., B. Ten Brink, and J.H.J. Huis In'T Veld. 1992. Selection of strains for probiotic use. Pages 209-224 *in:* Probiotics. The Scientific Basis. R. Fuller, ed. Chapman and Hall, London, UK.

Hesselman, K. and P. Åman. 1986. The effect of ß-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. Anim. Feed Sci. Technol. 15:83-93.

Hew, L.I., V. Ravindran, Y. Mollah, R.J. Gill and W.L. Bryden. 1995. Enzyme supplementation improves ileal amino acid digestibility values of wheat for broilers. Proc. Aust. Poult. Sci. Sym. 1995:189.

Hock, E., I. Halle, S. Matthes, and H. Jeroch. 1997. Investigations on the composition of the ileal and caecal microflora of broiler chicks in consideration to dietary enzyme preparation and zinc bacitracin in wheat-based diets. Agribiol. Res. 50:85-95.

Hofacre, C.L., R. Froyman, B. George, M.A. Goodwin, and J. Brown. 1998. Use of Aviguard, virginiamycin, or bacitracin MD against *Clostridium perfringens*-associated necrotizing enteritis. J. Appl. Poult. Res. 7:412-418.

Houghton, S.B., R. Fuller, and M.E. Coates. 1981. Correlation of growth depression of chicks with the presence of *Streptococcus faecium* in the gut. J. Appl. Bacteriol. 51:113-120.

Howard, M.D., D.T. Gordon, K.A. Garleb, and M.S. Kerley. 1995. Dietary fructooligosaccharide, xylooligosaccharide and gum arabic have variable effects on cecal and colonic microbiota and epithelial cell proliferation in mice and rats. J. Nutr. 125:2604-2609.

Hughes, R.J., M.Choct, A. Kocher, and R.J. van Barneveld. 2000. Effect of food enzymes on AME and composition of digesta from broiler chickens fed on diets containing non-starch polysaccharides isolated from lupin kernel. Br. Poult. Sci. 41:318-323.

Huisman, M.M.H., H.A. Schols, and A.G.J. Voragen. 2000. Glucuronoarabainoxylans from maize kernel cell walls are more complex than those from sorghum kernel cell walls. Carbohydr. Polym. 43:269-279.

Hume, M.E., D.J. Nisbet, C.M. Scanlan, D.E. Corrier, and J.R. DeLoach, 1995. Fermentation of radiolabelled substrates by batch cultures of caecal microflora maintained in a continuous-flow culture. J. Appl. Bacteriol. 78:677-683.

Hume, M.E., L.F. Kubena, R.C. Beier, A. Hinton, Jr., D.E. Corrier, and J.R. DeLoach, 1992. Fermentation of [^{14}C]lactose in broiler chicks by cecal anaerobes. Poultry Sci. 71:1464-1470.

Hungate, R.E. 1950. The anaerobic mesophilic cellulytic bacteria. Bacteriol. Rev. 14:1-49.

Hurwitz, S., U. Eisner, D. Dubrov, D. Sklan, G. Riesenfeld and A. Bar. 1979. Protein, fatty acids, calcium and phosphate absorption along the gastrointestinal tract of the young turkey. Comp. Biochem. Physiol. 62A:847-850.

Huyghebaert, G. and F.J. Schöner. 1999. Influence of storage and addition of enzyme on metabolisable energy content of wheat. 1. Impact of storage and enzyme addition. Arch. Geflügelk. 63:13-20.

Huyghebaert, G., T. Hastrup, W.D. Cowan, and P.B. Rasmussen. 1995. Impact of specific enzymes on the metabolisable energy of selected feedstuffs in broiler diets. Proc. Aust. Poult. Sci. Sym. 1995:130-134.

Irish, G.G., G.W. Barbour, H.L. Classen, R.T. Tyler and M.R. Bedford. 1995. Removal of the a-galactosides of sucrose from soybean meal using either ethanol extraction or exogenous a-galactosidase and broiler performance. Poultry Sci. 74:1484-1494.

Jehl, N. and T. Gidenne. 1996. Replacement of starch by digestible fibre in feed for the growing rabbit. 2. Consequences for microbial activity in the caecum and on incidence of digestive disorders. Anim. Feed Sci. Technol. 61:193-204.

Jensen, M.S., K.E. Bach Knudsen, J. Inborr, and K. Jakobsen. 1998. Effect of ß-glucanase supplementation on pancreatic enzyme activity and nutrient digestibility in piglets fed diets based on hulled and hulless barley varieties. Anim. Feed Sci. Technol. 72:329-345.

Jernigan, M.A., R.D. Miles, and A.S. Arafa. 1985. Probiotics in poultry nutrition - A review. World's Poult. Sci. J. 41:99-107.

Johnson, I.T., and J..M. Gee. 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport *in vitro*. Gut 22:398-403.

Johnson , I.T., J.M. Gee and R.R. Mahoney. 1984. Effect of dietary supplements of guar gum and cellulose on intestinal cell proliferation, enzyme levels and sugar transport in the rat. Br. J. Nutr. 52:477-487.

Johnson, I.T. and J.M. Gee. 1986. Gastrointestinal adaptation in response to soluble non-available polysaccharide in the rat. Br. J. Nutr. 55:497-505.

Jørgensen, H., X.Q. Zhao, K.E. Bach Knudsen, and B.O. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379-395.

Juven, B.J., R.J. Meinersmann, and N.J. Stern. 1991. A review. Antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. J. Appl. Bacteriol. 70:95-103.

Karasawa, Y., 1989. Ammonia production from uric acid, urea, and amino acids and its absorption from the ceca of the cockerel. J. Exp. Zool. Suppl.3:75-80.

Karasawa, Y. and M. Maeda. 1995. *In situ* degradation and absorption of [^{15}N]urea in chicken ceca. Comp. Biochem. Physiol. 111A:223-227.

Karasawa, Y., M. Okamoto and H. Kawai. 1988. Ammonia production from uric acid and its absorption from the caecum of the cockerel. Br. Poult. Sci. 29:119-124.

Kelly, B.J., N.D. Primm, C.L. Hofacre, and M.A. Goodwin. 1999. Comparison of a chicken origin competitive exclusion culture and a lyophilized probiotic to fresh turkey caecal material for efficacy against *Salmonella* colonization. Proc. 48th West. Poult. Dis. Conf. Vancouver, Canada. pp. 118.

Key, F.B. and J.C. Mathers. 1993a. Gastrointestinal responses of rats fed on white and wholemeal breads: complex carbohydrate digestibility and the influence of dietary fat content. Br. J. Nutr. 69:481-495.

Key, F.B. and J.C. Mathers. 1993b. Complex carbohydrate digestion and large bowel fermentation in rats given wholemeal bread and cooked haricot beans (*Phaseolus vulgaris*) fed in mixed diets. Br. J. Nutr. 69:497-509.

Kocher, A., M. Choct, R.J. Hughes and J. Broz. 2000. Effect of food enzymes on utilisation of lupin carbohydrates by broilers. Br. Poult. Sci. 41:75-82.

Kohmoto, T., F. Fukui, A. Takaku and T. Mitsuoka. 1991. Dose-response test of isomaltooligosaccharides for increasing faecal bifidobacteria. Agric. Biol. Chem. 55:2157-2159.

Kuo, T.M., J.F. van Middlesworth and W.J. Wolf. 1988. Content of raffinose oligosaccharides and sucrose in various plant seeds. J. Agric. Food Chem. 36:32-36.

Langendijk, P.S., F. Schut, G.J. Jansen, G.C. Raangs, G.R. Kamphuis, M.H.F. Wilkinson, and G.W. Welling. 1995. Quantitative fluorescence in situ hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl. Environ. Microbiol. 61:3069-3075.

Langhout, D.J., J.B. Schutte, C. Geerse, A.K. Kies, J. de Jong, and M.W.A. Verstegen. 1997. Effects on chick performance and nutrient digestibility of an endo-xylanase added to a wheat- and rye-based diet in relation to fat source. Br. Poult. Sci. 38:557-563.

Langhout, D. J. 1998. The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks. Ph.D. Thesis. Wageningen Agricultural University, TNO Nutrition and Food Research Institute, Department of Animal Nutrition and Physiology, Wageningen, The Netherlands.

Langhout, D.J., J.B. Schutte, P. van Leeuwen, J. Wiebenga and S. Tamminga. 1999. Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci. 40:340-347.

Langhout, D.J., J.B. Schutte, J. de Jong, M.W.A. Verstegen and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83:533-540.

Longstaff, M.A., A. Knox, and J.M. McNab, 1988. Digestibility of pentose sugars and uronic acids and their effect on chick weight gain and caecal size. Br. Poult. Sci. 29:379-393.

Lopez, H.W., M.A. Levrat, C. Guy, A. Messager, C. Demigne, and C. Remesy. 1999. Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. J. Nutr. Biochem. 10:500-509.

Mares, D.J. and B.A. Stone. 1973. Studies on wheat endosperm I. Chemical composition and ultrastructure of the cell walls. Aust. J. Biol. Sci. 26:793-812.

Mateos, G.G., J.L. Sell and J.A. Eastwood. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. Poultry Sci. 61:94-100.

McBee, R.H., 1989. Hindgut fermentations in nonavian species. J. Exp. Zool. Suppl.3:55-60.

McLelland, J., 1989. Anatomy of the avian cecum. J. Exp. Zool. Suppl.3:2-9.

McNab, J.M., 1973. The avian caeca: a review. World's Poult. Sci. J. 29:251-263.

Mead, G.C. 1989. Microbes of the avian cecum: types present and substrates utilized. J. Exp. Zool. Suppl.3:48-54.

Mead, G.C. 1997. Bacteria in the gastrointestinal tract of birds. Pages 216-240 *in:* Gastrointestinal Microbiology, Volume 2: Gastrointestinal Microbes and Host Interactions. R.I. Mackie, B.A. White and R.E. Isaacson, eds. Chapman and Hall, New York.

Mead, G.C. 2000. Microbial ecology of the digestive tract. Proc. XXI World's Poult. Cong. 2000:CD-ROM.

Mead, G.C. and B.W. Adams. 1975. Some observations on the caecal microflora of the chick during the first two weeks of life. Br. Poult. Sci. 16:169-176.

Mohan, B., R. Kadirvel, A. Natarajan, and M. Bhaskaran. 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. Br. Poult. Sci. 37:395-401.

Mohan, B., R. Kadirvel, M. Bhaskaran, and A. Natarajan. 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. Br. Poult. Sci. 36:799-803.

Mollah, Y., W.L. Bryden, I.R. Wallis, D. Balnave and E.F. Annison. 1983. Studies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. Br. Poult. Sci. 24:81-89.

Monsan, P.F. and F. Paul. 1995. Oligosaccharide feed additives. Pages 233-245 *in:* Biotechnology in Animal Feeds and Animal Feeding. R.J. Wallace and A. Chesson, ed. VCH, Weinheim, Germany.

Moran, E.T. 1982. Comparative Nutrition of Fowl & Swine: The Gastrointestinal Systems. E.T. Moran. University of Guelph.

Moretó, M., and J.M. Planas, 1989. Sugar and amino acid transport properties of the chicken ceca. J. Exp. Zool. Suppl.3:111-116.

Morgan, A.J. and M.R. Bedford. 1995. Advances in the development and application of feed enzymes. Proc. Aust. Poult. Sci. Sym. 1995:109-115.

Morishita, Y., R. Fuller, and M.E. Coates, 1982. Influence of dietary lactose on the gut flora of chicks. Br. Poult. Sci. 23:349-359.

Moss, R., 1989. Gut size and the digestion of fibrous diets by tetraonid birds. J. Exp. Zool. Suppl. 3:61-65.

Muramatsu, T., O. Takasu, M. Furuse, and J. Okumura. 1988. Effect of diet type on enhanced intestinal protein synthesis by the gut microflora in the chick. J. Nutr. 118:1068-1074.

Muramatsu, T., H. Kodama, T. Morishita, M. Furuse, and J. Okumura, 1991. Effect of intestinal microflora on digestible energy and fiber digestion in chickens fed a high-fiber diet. Am. J. Vet. Res. 52:1178-1181.

Muramatsu, T., J. Takemura, and J. Okumura. 1993. Acetic acid is not involved in enhanced intestinal protein synthesis by the presence of the gut microflora in chickens. Comp. Biochem. Physiol. 105A:543-548.

Muramatsu, T., S. Nakajima, and J. Okumura, 1994. Modification of energy metabolism by the presence of gut microflora in the chicken. Br. J. Nutr. 71:709-717.

Nisbet, D.J., D.E. Corrier, and J.R. DeLoach. 1993. Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on *Salmonella typhimurium* colonization in broiler chicks. Avian Dis. 37:528-535.

Noy, Y. and D. Sklan. 1995. Digestion and absorption in the young chick. Poultry Sci. 74:366-373.

Obst, B.S., and J.M. Diamond, 1989. Interspecific variation in sugar and amino acid transport by the avian cecum. J. Exp. Zool. Suppl. 3:117-126.

Orban, J.I., J.A. Patterson, A.L. Sutton, and G.N. Richards. 1997. Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations of broiler chickens. Poultry Sci. 76:482-490.

Owings, W.J., D.L. Reynolds, R.J. Hasiak, and P.R. Ferket. 1990. Influence of dietary supplementation with *Streptococcus faecium* M-74 on broiler body weight, feed conversion, carcass characteristics, and intestinal microbial colonization. Poultry Sci. 69:1257-1264.

Palmu, L., and I. Camelin. 1997. Use of competitive exclusion in broilers to reduce the level of *Salmonella* contamination on the farm and at the processing plant. Poultry Sci. 76:1501-1505.

Patterson, J.A., J.I. Orban, A.L. Sutton, and G.N. Richards. 1997. Selective enrichment of bifidobacteria in the intestinal tract of broilers by thermally produced kestoses and effect on broiler performance. Poultry Sci. 76:497-500.

Pawlik, J.R., A.I. Fengler, and R.R. Marquardt. 1990. Improvement of the nutritional value of rye by the partial hydrolysis of the viscous water-soluble pentosans following water-soaking or fungal enzyme treatment. Br. Poult. Sci. 31:525-538.

Petersen, S., J. Wiseman and M. Bedford. 1999. Effects of age and diet on the viscosity of intestinal contents in broiler chicks. Br. Poult. Sci. 40:364-370.

Pettersson, D. and P. Åman. 1989. Enzyme supplementation of a poultry diet containing rye and wheat. Br. J. Nutr. 62:139-149.

Petterson, D., H. Graham and P. Åman. 1990. Enzyme supplementation of broiler chicken diets based on cereals with endosperm cell walls rich in arabinoxylans and mixed-linked ß-glucans. Anim. Prod. 51:201-207.

Planas, J.M., R. Ferrer, and M. Moretó. 1987. Relation between a-methyl-D-glucoside influx and brush border surface area in enterocytes from chicken cecum and jejunum. Pflügers Arch. 408:515-518.

Planas, J.M., M.C. Villá, R. Ferrer, and M. Moretó. 1986. Hexose transport by chicken cecum during development. Pflügers Arch. 407:216-220.

Posner, E.S. 2000. Wheat. Pages 1- 29 *in:* Handbook of Cereal Science and Technology, Second Edition, Revised and Expanded. K. Kulp and J.G. Ponte, Jr., ed. Marcel Dekker, Inc. New York, NY.

Potter, L.M., M.W. Stutz, and L.D. Matterson. 1965. Metabolizable energy and digestibility coefficients of barley for chicks as influenced by water treatment or by presence of fungal enzyme. Poultry Sci. 44:565-573.

Pylot, S.J., J.J. McKinnon, T.A. McAllister, A.F. Mustafa, J. Popp, and D.A. Christensen. 2000. Canola screenings as a fiber source in barley-based feedlot diets: Effects on rumen fermentation and performance of steers. Can. J. Anim. Sci. 80:161-168.

Redig, P.T., 1989. The avian ceca: Obligate combustion chambers or facultative afterburners? - The conditioning influence of diet. J. Exp. Zool. Suppl. 3:66-69.

Riesenfeld, G., D. Sklan, A. Bar, U. Eisner and S. Hurwitz. 1980. Glucose absorption and starch digestion in the intestine of the chicken. J. Nutr. 110:117-121.

Remington, T.E., 1989. Why do grouse have ceca? A test of the fiber digestion theory. J. Exp. Zool. Suppl. 3:87-94.

Roberfroid, M. 1993. Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. Crit. Rev. Food Sci. Nutr. 33:103-148.

Rogel, A.M., E.F. Annison, W.L. Bryden and D. Balnave. 1987. The digestion of wheat starch in broiler chickens. Aust. J. Agric. Res. 38:639-649.

Rosen, G.D. 2001. Multi-factorial efficacy evaluation of alternatives to antimicrobials in pronutrition. Pp. 24-25. *In:* Proceedings of the Spring Meeting, World's Poultry Science Association, UK Branch. University of York, April 10-11, 2001.

Rotter, B.A., R.R. Marquardt, W. Guenter, C. Biliaderis and C.W. Newman. 1989. In vitro viscosity measurements of barley extracts as predictors of growth responses in chicks fed barley-based diets supplemented with a fungal enzyme preparation. Can. J. Anim. Sci. 69:431-439.

Rotter, B.A., O.D. Friesen, W. Guenter and R.R. Marquardt. 1990. Influence of enzyme supplementation on the bioavailable energy of barley. Poultry Sci. 69:1174-1181.

Salanitro, J.P., I.G. Blake, P.A. Muirhead, M. Maglio, and J.R. Goodman. 1978. Bacteria isolated from the duodenum, ileum and cecum of young chicks. Appl. Environ. Microbiol. 35:782-790.

Salih, M.E., H.L. Classen, and G.L. Campbell. 1991. Response of chickens fed on hull-less barley to ß-glucanase at different ages. Anim. Feed Sci. Technol. 33:139-149.

SAS Institute. 1989. SAS/STAT® User's Guide. Version 6, Fourth Edition. SAS Institute Inc., Cary, NC.

Savage, T.F., E.I. Zakrzewska, J.R. Andreasen Jr. 1997. The effects of feeding mannan oligosaccharide supplemented diets to poults on performance and the morphology of the small intestine. Poster presented at Southern Poultry Science, Atlanta, Georgia, January, 1997.

Savory, C.J., 1992a. Gastrointestinal morphology and absorption of monosaccharides in fowls conditioned to different types and levels of dietary fibre. Br. J. Nutr. 67:77-89.

Savory, C.J., 1992b. Enzyme supplementation, degradation and metabolism of three U-^{14}C-labelled cell-wall substrates in the fowl. Br. J. Nutr. 67:91-102.

Savory, C.J., 1992c. Metabolic fates of U-^{14}C-labelled monosaccharides and an enzyme-treated cell-wall substrate in the fowl. Br. J. Nutr. 67:103-114.

Savory, C.J. and A.I. Knox, 1991. Chemical composition of caecal contents in the fowl in relation to dietary fibre level and time of day. Comp. Biochem. Physiol. [A] 100:739-743.

Schneitz, C. and M. Häkkinen. 1998. Comparison of two different types of competitive exclusion products. Lett. Appl. Microbiol. 26:338-341.

Schutte, J.B. 1990. Nutritional implications and metabolizable energy value of D-xylose and L-arabinose in chicks. Poultry Sci. 69:1724-1730.

Schutte, J.B., P. van Leeuwen, and W.J. Lightendonk. 1991. Ileal digestibility and urinary excretion of D-xylose and L-arabinose in ileostomized adult roosters. Poultry Sci. 70:884-891.

Schutte, J.B., J. de Jong, E.J. van Weerden, and M.J. van Baak. 1992. Nutritional value of D-xylose and L-arabinose for broiler chicks. Br. Poult. Sci. 33:89-100.

Scott, T.A., F.G. Silversides, H.L. Classen, M.L. Swift, M.R. Bedford, and J.W. Hall. 1998a. A broiler chick bioassay for measuring the feeding value of wheat and barley in complete diets. Poultry Sci. 77:449-455.

Scott, T.A., F.G. Silversides, H.L. Classen, M.L. Swift, and M.R. Bedford. 1998b. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent digestible energy of wheat and barley. Poultry Sci. 77:456-463.

Scott, T.A., F.G. Silversides, H.L. Classen, M.L. Swift, and M.R. Bedford. 1998c. Effect of cultivar and environment on the feeding value of Western Canadian wheat and barley samples with and without enzyme supplementation. Can. J. Anim. Sci. 78: 649-656.

Scott, T.A., F.G. Silversides, H.L. Classen, M.L. Swift, and M.R. Bedford. 1999. Prediction of the performance of broiler chicks from apparent metabolizable energy and protein digestibility values obtained using a broiler chick bioassay. Can. J. Anim. Sci. 79:59-64.

Selvendran, R.R. 1984. The plant cell wall as a source of dietary fiber: chemistry and structure. Am. J. Clin. Nutr. 39:320-337.

Shehata, A.T., J. Lerner, and D.S. Miller. 1981. Development of brush-border membrane hexose transport system in chick jejunum. Am. J. Physiol. 240:G102-G108.

Shelton, D.R. and W.J. Lee. 2000. Cereal Carbohydrates. Pages 385-415 *in:* Handbook of Cereal Science and Technology, Second Edition, Revised and Expanded. K. Kulp and J.G. Ponte, Jr., ed. Marcel Dekker, Inc. New York, NY.

Sissons, J.W. 1989. Potential for probiotic organisms to prevent diarrhoea and promote digestion in farm animals - A review. J. Sci. Food Agric. 49:1-13.

Slominski, B.A. 1994. Hydrolysis of galactooligosaccharides by commercial preparations of a-galactosidase and b-fructofuranosidase: potential for use as dietary additives. J. Sci. Food Agric. 65:323-330.

Slominski, B.A. and L.D. Campbell. 1990. Non-starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53:175-184.

Slominski, B.A., L.D. Campbell and W. Guenter. 1994. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci. 73:156-162.

Smith, H.W. 1965. The development of the flora of the alimentary tract in young animals. J. Pathol. Bacteriol. 90:495-513.

Smits, C.H.M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52:203-221.

Smits, C.H.M., A. Veldman, M.W.A. Verstegen and A.C. Beynen. 1997. Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr. 127:483-487.

Smits, C.H.M., A. Veldman, H.J. Verkade and A.C. Beynen. 1998. The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine. Poultry Sci. 77:1534-1539.

Snel, J., P.P. Heinen, H.J. Blok, R.J. Carman, A.J. Duncan, P.C. Allen, and M.D. Collins. 1995. Comparison of 16S rRNA sequences of segmented filamentous bacteria isolated from mice, rats, and chickens and proposal of "*Candidatus* arthromitus". Int. J. Syst. Bacteriol. 45:780-782.

Soita, H.W. 2001. The influence of forage particle size on rumen metabolic responses and nutrient utilization. Ph.D Thesis. University of Saskatchewan, Saskatoon, SK Canada.

Son, J.H. and Y. Karasawa. 2000. Effect of removal of caecal contents on nitrogen utilisation and nitrogen excretion in caecally ligated chickens fed on a low protein diet supplemented with urea. Br. Poult. Sci. 41:69-71.

Son, J.H., Y. Karasawa and K.H. Nahm. 2000. Effect of caecectomy on growth, moisture in excreta, gastrointestinal passage time and uric acid excretion in growing chicks. Br. Poult. Sci. 41:72-74.

Stavric, S., T.M. Gleeson, B. Buchanan, and B. Blanchfield. 1992. Experience of the use of probiotics for *Salmonellae* control in poultry. Lett. Appl. Microbiol. 14:69-71.

Stavric, S. and E.T. Kornegay. 1995. Microbial probiotics for pigs and poultry. Pages 205-231 *in:* Biotechnology in Animal Feeds and Animal Feeding. R.J. Wallace and A. Chesson, ed. VCH, Weinheim, Germany.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. A biometrical approach, 2nd Ed. McGraw-Hill Publishing Co., New York, NY.

Steenfeldt, S, A. Müllertz and J.F. Jensen. 1998a. Enzyme supplementation of wheat-based diets for broilers. 1. Effect on growth performance and intestinal viscosity. Anim. Feed Sci. Technol. 75:27-43.

Steenfeldt, S, M. Hammershøj, A. Müllertz and J.F. Jensen. 1998b. Enzyme supplementation of wheat-based diets for broilers. 2. Effect on apparent metabolisable energy content and nutrient digestibility. Anim. Feed Sci. Technol. 75:45-64.

Strong, T.R., P.R. Reimer, and E.J. Braun, 1989. Avian cecal microanatomy: A morphometric comparison of two species. J. Exp. Zool. Suppl. 3:10-20.

Sudo, S.Z., and G.E. Duke, 1980. Kinetics of absorption of volatile fatty acids from the ceca of domestic turkeys. Comp. Biochem. Physiol. [A] 67:231-237.

Theander, O., E. Westerlund, P. Åman and H. Graham. 1989. Plant cell walls and monogastric diets. Anim. Feed Sci. Technol. 23:205-225.

Theander, O., and P. Åman, 1979. The chemistry, morphology, and analysis of dietary --fiber components. Pages 215-244 *in*: Dietary Fibers: Chemistry and Nutrition. G.E. Inglett and I. Falkehag, ed. Academic Press, London.

Theander, O., E. Westerlund, and P. Åman, 1993. Structure and components of dietary fiber. Cereal Foods World. 38:135-141.

Thomas, D.H., and E. Skadhauge, 1988. Transport function and control in bird caeca. Comp. Biochem. Physiol. [A] 90:591-596.

Thomas, D.H., and E. Skadhauge, 1989. Water and electrolyte transport by the avian ceca. J. Exp. Zool. Suppl. 3:95-102.

Trowell, H., D.A.T. Southgate, T.M.S. Wolever, A.R. Leeds, M.A. Gussell, and D.J.A. Jenkins, 1976. Dietary fibre redefined. Lancet. 1:967.

Vahjen, W., K. Glässer, K. Schäfer and O. Simon, 1998. Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J. Agric. Sci. 130:489-500.

Van der Klis, J.D. 1993. Physicochemical chyme conditions and mineral absorption in broilers. Ph.D. Thesis, Spelderholt Report 595.

Van der Klis, J.D. and A. van Voorst. 1993. The effect of carboxy methyl cellulose (a soluble polysaccharide) on the rate of marker excretion from the gastrointestinal tract of broilers. Poultry Sci. 72:503-512.

Van der Klis, J.D., A. van Voorst, and C. van Cruyningen. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the physico-chemical conditions in the gastrointestinal tract of broilers. Br. Poult. Sci. 34: 971-983.

Van der Klis, J.D., C. Kwakernaak, and W. de Wit. 1995. Effects of endoxylanase addition to wheat-based diets on physico-chemical chyme conditions and mineral absorption in broilers. Anim. Feed Sci. Technol. 51:15-27.

Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. pp. 374. O & B Books, Corvallis, OR.

Veldman, A. and H.A. Vahl. 1994. Xylanase in broiler diets with differences in characteristics and content of wheat. Br. Poult. Sci. 35:537-550.

Veldman, A., W.A.G. Veen, D. Barug and P.A. van Paridon. 1993. Effect of a-galactosides and a-galactosidase in feed on ileal piglet digestive physiology. J. Anim. Physiol. Anim. Nutr. 69:57-65.

Villamide, M.J., J.M. Fuente, P. Perez de Ayala, and A. Flores. 1997. Energy evaluation of eight barley cultivars for poultry: effect of dietary enzyme addition. Poultry Sci. 76:834-840.

Vinardell, M.P., M.T. Lopera, and M. Moretó. 1986. Absorption of 3-oxy-methyl-D-glucose by chicken cecum and jejunum *in vivo.* Comp. Biochem. Physiol. [A] 85:171-173.

Vukic Vranjes, M. and C. Wenk. 1996. Influence of *Trichoderma viride* enzyme complex on nutrient utilization and performance of laying hens in diets with and without antibiotic supplementation. Poultry Sci 75:551-555.

Wagh, P.V. and P.E. Waibel. 1967a. Metabolism of L-arabinose and D-xylose by chicks. J. Nutr. 92:491-496.

Wagh, P.V. and P.E. Waibel. 1967b. Alimentary absorption of L-arabinose and D-xylose in chicks. Proc. Soc. Exp. Biol. Med. 124:421-424.

Wagner, D.D. and O.P. Thomas. 1978. Influence of diets containing rye or pectin on the intestinal flora of chicks. Poultry Sci. 57:971-975.

Ward, A.T. and R.R. Marquardt. 1983. The effect of saturation, chain length of pure triglycerides, and age of bird on the utilization of rye diets. Poultry Sci. 62:1054-1062.

Wiggins, H.S. 1984. Nutritional value of sugars and related compounds undigested in the small gut. Proc. Nutr. Soc. 43:69-75.

Willingham, H.E., K.C. Leong, L.S. Jensen, and J. McGinnis. 1960. Influence of geographical area of production on response of different barley samples to enzyme supplements or water treatment. Poultry Sci. 39:103-108.

Yasar, S. and J.M. Forbes. 1999. Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. Br. J. Nutr. 40:65-76.

Yule, M.A. and M.F. Fuller. 1992. The utilization of orally administered D-xylose, L-arabinose and D-galacturonic acid in the pig. Int. J. Food Sci. Nutr. 43:31-40.