Stellingen

1. De waarde van de *in vitro* viscositeit als indicator voor het voorspellen van de voederwaarde van tarwe bij kuikens is twijfelachtig. *dit proefschrift*

2. Bij vleeskuikens is de relatie tussen de viscositeit van het supernatant van de dunne darminhoud en de productieresultaten niet duidelijk. *dit proefschrift*

3. De mate van deconjugatie van galzouten in de dunne darminhoud bij kuikens is een betere maat voor de microbiële activiteit in de dunne darm dan de pH, de concentratie aan melkzuur of die van vluchtige vetzuren in de dunne darminhoud. *dit proefschrift*

4. De activiteit en soort darmflora zijn in hoge mate bepalend voor de darmgezondheid en de productieresultaten van vleeskuikens. *dit proefschrift*

5. Verandering in de samenstelling van de huidige Nederlandse vleeskuikenvoeders kan bijdragen aan een betere gezondheid en productieresultaten van vleeskuikens. *dit proefschrift*

6. De productie en samenstelling van vluchtige vetzuren in het maagdarmkanaal van het varken is nutriënt afhankelijk.
   proefschrift J.B. Schutte (1991), Wageningen

7. Onderzoek naar het enteraal zenuwstelsel met de cavia als model zal pas waarde hebben voor andere diersoorten als duidelijk is hoe representatief het darmkanaal van de cavia is voor andere diersoorten.
   proefschrift I.W.M. Schutte (1998), Wageningen

8. Dat mensen en gezelschapsdieren ongebreideld ammoniak mogen produceren terwijl dit bij landbouwhuisdieren niet wordt geaccepteerd is een vorm van discriminatie.

9. Verschillende onderzoekers hebben een fundamenteel verschillende voorstelling van het begrip fundamenteel onderzoek.

10. De verkeersonveiligheid rond scholen tijdens het wegbrengen en halen van de kinderen wordt voor een belangrijk deel veroorzaakt door de ouders zelf.

11. Door de toename aan elektrische apparatuur in de huishouding vergt het verrichten van huishoudelijk werk steeds meer geestelijke i.p.v. lichamelijke inspanning.

12. Proefschriften krijgen in de landelijke pers ten onrechte meestal minder aandacht dan de bijbehorende stellingen.

Proefschrift D.J. Langhout
The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks.
Wageningen, 23 oktober 1998.

The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks

Promotoren: Dr. ir. M.W.A. Verstegen
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The role of the intestinal flora as affected by non-starch polysaccharides in broiler chicks

D. J. Langhout

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus,
van de Landbouwuniversiteit Wageningen,
Dr. C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 23 oktober 1998
des namiddags te half twee in de aula

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It is well established that the non-starch polysaccharides (NSP) of wheat, rye and barley have anti-nutritive properties in broiler chicks. The water-soluble fraction of these NSP are assumed to be primary responsible for the anti-nutritive activity, producing depression in digestibility of nutrients and performance. This study was undertaken to investigate the role of the intestinal microflora on the anti-nutritive properties of water-soluble NSP by using citrus pectin as model substrate. Citrus pectin is a highly fermentable NSP fraction of a viscous nature. Inclusion of high-methylated citrus pectin (HMC) in diets of young chicks resulted in a decreased nutrient digestibility and performance. This was associated with an increase of the microbial activity in the small intestine. In addition, ileal composition of the microflora and the morphology of the small intestinal wall was changed. Furthermore, degradation of conjugated bile acids was increased significantly by including HMC in the diet. The effect of low-methylated citrus pectin (LMC) on these parameters was less pronounced than with HMC. When the HMC diet was fed to germ-free chicks, digestibility of nutrients and performance were hardly affected. It was concluded that the intestinal microflora mediates the magnitude of the anti-nutritive properties of water-soluble NSP in broiler chicks.

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Voor Irma

Voor Nicole, Sylvie en Judith

Voor mijn ouders

Contents

General introduction

General introduction

Worldwide, the poultry industry has gone through an enormous development and expansion during the second half of this century. Growth of broilers has been markedly increased (with 85 to 90%), and feed utilization has been improved (with 45 to 50%; Havenstein *et al.*, 1994[a,b]). This development of the poultry industry, however, has also a reverse side. Modern chicks have a poor immunocompetence, and exhibit a propensity for skeletal and metabolic disorders (Havenstein *et al.*, 1994[a,b]). These metabolic disorders are assumed to be related, at least in part, to disturbances in the gut ecosystem.

The gut ecosystem of broiler chicks is a complex ecosystem, which can be relatively easily disturbed in young broiler chicks. It has been estimated that in broiler chicks up to 10^{14} bacteria can be found in the gut lumen (Mead, 1989). Thus, bacteria in the digestive tract can play an important role in metabolism in the gut lumen (Savage, 1986; Fuller and Cole, 1988). However, knowledge of the significance of the gut microflora to the nutrition of chicks is scarce.

In broiler diets, the carbohydrate fraction is the primary source affecting intestinal microbial activity. The effect of carbohydrates on intestinal microbial activity depends mainly on the type of carbohydrate. Literature data indicate that the water-soluble NSP fraction increases microbial activity in the intestinal tract of broiler chicks (Wagner and Thomas, 1978; Smits, 1996). The insoluble NSP fraction, on the other hand, may reduce microbial activity by increasing the passage rate (Robertson, 1988).

It has been suggested that the water-soluble NSP present in barley, rye and wheat reduce performance in broiler chicks (Antoniou *et al.*, 1981; White *et al.*, 1981; Fengler and Marquardt, 1988; Choct and Annison, 1990). The negative effect of water-soluble NSP on performance appears to be mediated by the intestinal microflora. Water-soluble NSP are known to reduce digestibility of nutrients in the small intestine. The microflora in the small intestine is believed to interfere with enzymatic hydrolysis and absorption of nutrients when water-soluble NSP are included in the diet. The mechanism by which bacteria affect nutrient utilization is still under discussion. It has been hypothesized that the intestinal microflora may influence fat digestion through transformation of bile acids. In addition, protein and starch digestion may be impaired through increased competition between the host and the microflora. In addition, absorption of nutrients may be reduced through a change in gut morphology. The latter will also reduce utilization of nutrients through an increase in cell turnover of the gut wall.

Water-soluble NSP are of a viscous nature and can be fermented by the gut microflora. Previous studies on the anti-nutritive effects of water-soluble NSP were mainly focused on their viscous properties (Van der Klis, 1993; Smits, 1996). Water-soluble NSP not only have viscous properties, but are generally also fermented in the intestinal tract of broiler chicks. Therefore, it is likely that water-soluble NSP affect microbial activity in the intestinal tract. With regard to the latter, the fermentability of water-soluble NSP might be an important factor.

Aim and outline of this thesis

The objective of this thesis is to determine the effect of the intestinal microflora as affected by chemical and physical properties of water-soluble NSP on the broiler performance and nutrient digestibility. Information presented in this thesis may contribute towards a better understanding of the role of the microflora in the gastrointestinal tract on broiler nutrition.

As a start, a literature review described the effects of carbohydrates on the microbial activity in the intestinal tract and subsequent effects on nutrient digestion in broiler diets (Chapter 1). The increase in viscosity of digesta was still thought to be the primary mechanism by which water-soluble NSP exert their anti-nutritive effects. Therefore, the first studies (Chapter 2) were carried out to test the hypothesis that it is not the digesta viscosity alone that explains the anti-nutritive effects of these NSP fractions. Pectin, a highly fermentable NSP fraction of a viscous nature, was then selected to examine the effect of viscous water-soluble NSP on microbial composition and microbial activity in broiler chicks. Morphology of the intestinal wall and fat digestibility are assumed to be affected by the microflora. In Chapter 3, the anti-nutritive effects of different pectin products, with similar and different viscous properties, are discussed. In Chapter 4, the effects of high- and low-methylated citrus pectin on the composition of the ileal microflora, the morphology of the ileal gut wall and nutrient digestibility are discussed. In order to be able to relate the results of both pectin products to practice, also rye and wheat were included in a similar study (Chapter 5). This chapter describes a study in which the effect of dietary viscous water-soluble NSP on microbial activity and composition was investigated. In addition, the effect of the microflora on bile acids was determined to obtain a better understanding of the role of the microflora in fat digestion. It is emphasized that the anti-nutritive effects of the water-soluble NSP fractions on broiler performance and digestion of nutrients is mainly mediated by the gut microflora.

This hypothesis was checked with germ-free chicks (Chapter 6). Finally, the main conclusions are summarized and some practical implications are discussed (General Discussion).

References

Antoniou, T., Marquardt, R.R. & Cansfield, E. (1981) Isolation, partial characterization and anti-nutritional activity of a factor (pentosans) in rye grain. *Journal of Agriculture and Food Chemistry* 29: 1240-1247.

Choct, M. & Annison, G. (1990) Antinutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

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

Fuller, R. & Cole, C.B. (1988) The scientic basis of the probiotic concept. In: *Probiotics - Theory and Applications* (Stark, B.A. & Wilkinson, J.M., eds.) Chalcombe Publications, Marlow, pp. 1-14.

Havenstein, G.B., Ferkel, P.R., Scheideler, S.E. & Larson, B.T. (1994^a) Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* 73: 1785-1794.

Havenstein, G.B., Ferkel, P.R., Scheideler, S.E. & Rives, D.V. (1994^b) Carcass composition, and yield of 1991 vs 1957 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* 73: 1795-1804.

Mead, G.C. (1989) Microbes of the avian cecum: types present and substrates utilized. *The Journal of Experimental Zoology* Suppl. 3: 48-54.

Robertson, J.A. (1988) Physicochemical characteristics of food and the digestion of starch and dietary fibre during the gut transit. *Proceedings of the Nutrition Society* 43: 83-88.

Savage, D.C. (1986) Gastro-intestinal microflora in mammalian nutrition. *Annual Review Nutrition* 6: 155-178.

Smits, C.H.M. (1996) Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. PhD thesis, Agricultural University Wageningen, the Netherlands.

Van der Klis, J.D. (1993) Physico-chemical chyme conditions and mineral absorption in broilers. PhD thesis, Agricultural University Wageningen, the Netherlands.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-875.

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1981) The viscosity interaction of barley ß-glucan with *Trichoderma viride* cellulase in the chick intestine. *Poultry Science* 62: 853-862.

Chapter 1

Effect of carbohydrates on microbial activity in the intestinal tract and subsequent effects on nutrient digestion in broiler chicks

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Effect of carbohydrates on microbial activity in the intestinal tract and subsequent effects on nutrient digestion in broiler chicks

D.J. Langhout

Introduction

Worldwide, the poultry industry has undergone an enormous development and expansion during the second half of this century. The production of poultry meat is highly specialized and intensified. In addition, broiler stocks are selected on the basis of growth. As a result, the growth period of broilers has been reduced by one day per year during the past decades. Besides, feed utilization has improved markedly. However, this development and expansion in poultry meat production has also a negative side. To-day's chicks eat near gut capacity, have poor immunocompetence, and exhibit a propensity for skeletal and metabolic disorders (Havenstein *et al.*, 1994[a,b]). These skeletal and metabolic disorders cause significant economic losses in broiler production. These metabolic disorders are assumed to be related, at least in part, to disturbances in the ecosystem of the digestive tract.

It is a generally accepted notion that a relation exists between intestinal microflora and health status of the host animal (Ferket, 1991; Brown, 1996). Many complex bacterial control mechanisms are involved to provide a balanced composition of the microflora in order to protect the animal against invading pathogens and to obtain optimal nutrient utilization. A balance between normal habitants of the intestinal tract has to be created in healthy animals. However, in present-day farming systems numerous environmental and dietary factors prevent young animals from obtaining a balanced microbial ecosystem.

The role of nutrition by the intestinal microflora of poultry has not been fully capitalized upon because of the general assumption that poultry have a 'simple' digestive tract and that their microflora contributes little to general nutrition. This does not, however, exclude its importance for other aspects. Nowadays broiler diets are characterized as 'high-energy low-crude-fibre diets'. The common energy sources in practical broiler diets are carbohydrates and fat. Evidence is accumulating that the microbial activity in the digestive tract is mainly affected by the source of dietary carbohydrate. This review will focus on the effects of carbohydrates on the microbial activity in the digestive tract of broilers in relation to nutrient and energy digestion.

Characterization of gut microflora in broilers

Non-ruminant animals have a gastrointestinal bacterial population that is mainly active further down the gastrointestinal tract than in ruminants. In addition, their capacity to degrade feed components indigestible to the host's enzymes is smaller than in ruminants. However, also the intestinal population in non-ruminants varies widely in composition and activity (Drasar, 1988), and in the distribution of the microflora in different regions of the intestinal tract (Rowland *et al.*, 1985). Presumably this variation results from different conditions in the digestive tract, which are affected for example by diet composition, dry matter content, and acid and alkali secretion (Savage, 1983). The consequences of the differences in gut microflora among species are hard to assess because of a lack of knowledge. This should be kept in mind wherever data on species other than poultry are referred to in this review.

In poultry, fermentation occurs mainly in the caeca. Relative to other parts of the intestinal tract of poultry, the caeca provide a stable environment for micro-organisms and, as a result, contain the largest and most complex ecosystem. However, considerable microbial activity is found also in the small intestine. Fermentation of nutrients in poultry may already start in the crop. Bayer *et al.* (1978) reported that lactic and acetic acid were the predominant acids in the crop when broiler chicks were fed on either high- or low-fibre diets. Therefore, it is likely that fermentation occurs in all sections of the digestive tract.

The digestion and absorption of nutrients by the animal depends on the rate of hydrolysis by the animal's enzymes and the activity of the microflora. The small intestine is the site where most of the hydrolysis and absorption of nutrients takes place. Therefore, changes in microbial activity, particularly in the small intestine, may influence nutrient digestibility. However, relatively little is known about the effects of the microflora on digestion and absorption of nutrients.

Development of the gut microflora in broilers

There are many different bacterial species present in the gastrointestinal tract of poultry (Table 1). It is usually that the intestinal tract of one-day-old chicks is sterile; however, within a few hours after hatching appreciable numbers of bacteria can be detected. In the small intestine, the bacterial population appears to be established within approximately 2 weeks (Ochi *et al.*, 1964; Smith, 1965). In contrast with this, it takes much longer for the caecal ecosystem to develop (Ochi *et al.*, 1964; Smith, 1965; Barnes *et al.*, 1972; Mead and Adams, 1975). Barnes *et al.* (1972) have shown that it takes even up to 6 or 7 weeks of age in poultry before the microbial population in the caeca has fully developed.

Table 1. *Common bacterial species in the intestinal tract of poultry.*

Gram-positive bacteria	Gram-negative bacteria
Aerobes	*Facultative anaerobes*
Streptococcus faecium	Escherichia coli
Streptococcus faecalis	Enterobacteriaceae spp.
Micrococcus spp.	Enterobacter spp.
Lactobacillus acidophilus	
Lactobacillus fermentum	*Anaerobes*
Lactobacillus salivaris	Gemminger formicilis
Lactobacillus spp.	
Bifidobacterium bifidum	*Strictly anaerobes*
Eubacteria spp.	Bacteriodaceae spp.
	Fusobacteria spp.
Facultative anaerobes	Bacteroides spp.
Staphylococcus spp.	
Anaerobes	
Ruminococcus obeum	
Clostridium perfringens	
Clostridium beyerinckii	
Clostridium spp.	

Adapted from Barnes *et al.*, 1972; Salanitro *et al.*, 1974, 1978; Mead, 1989.

Colonization by bacteria in the intestinal tract is the ability of bacteria to establish themselves in the digestive tract. Normally, bacteria are in equilibrium with each other and with the host animal. There is a constant selection of bacteria which can grow and colonize in the intestinal tract to produce a microflora specific to the host species. This selection of bacteria is partly of a chemical nature due to inhibitory agents such as volatile fatty acids, bile acids, hydrogen sulfide (Fuller, 1984) and immunoglobulins. When bacteria are able to survive these inhibitory effects, they must contend with the constant flushing effects resulting from peristaltic movements from the anterior regions of the gut. Bacteria remain in the intestinal tract in two ways (Ewing and Cole, 1994):

- by attachment to the epithelial cells lining the intestine
- by growing at a rate faster than the rate at which they are being removed by peristalsis (Fuller and Cole, 1988)

There is a considerable variation in microbial colonization between different regions of the intestinal tract. This may be due to differences in physical and chemical conditions such as pH, osmolality and substrate availability. Each bacterial species requires its own conditions to grow. Consequently, different bacterial species are found in different parts of the intestinal tract. In the crop and stomach, mainly Gram-positive bacteria such as *Lactobacilli* and *Streptococci* are found (Ochi *et al.*, 1964; Smith, 1965; Fuller and Turvey, 1971).

The predominant species in the small intestine of poultry are aerobes, Gram-positive *Enterococci*, *Lactobacilli* and *Bifidobacteria*, facultative anaerobes, Gram-negative *Coliform* bacteria and strictly anaerobes, Gram-positive *Clostridia* and Gram-negative *Bacteroides*. Gram-negative bacteria such as *Bacteroides* and *Fusobacteria* are found mainly in the caeca (Barnes *et al.*, 1972; Mead, 1989).

The diversity in bacterial species in the intestinal tract is one of the most important factors for a stable ecosystem in the intestinal tract. When the number of bacterial species in the intestinal tract is high, the intestinal ecosystem is considered to be more stable (Mead, 1989). Until the bacterial population is fully established, young animals have less bacterial species in the intestinal tract than adult birds. This implies that the balance in the ecosystem can be more easily disturbed in young than in adult birds (Mead, 1989).

Environmental differences between young and adult birds may also influence the growth of bacterial species in the intestinal tract. A low pH is bacteriostatic for Gram-negative microorganisms in the intestinal tract. The pH in the lower part of the intestinal tract is mainly related to the concentrations of lactic acid and volatile fatty acids (VFA). Lactic acid and VFA are fermentation products of bacteria. Bacterial counts are still limited in young animals. As a consequence, the pH in the intestine, in particular in the lower part of the intestinal tract, is higher in young birds than in adult poultry (Cummingham, 1989). Thus, in young birds inhibition of Gram-negative bacteria in the lower part of the gastrointestinal tract may be less than in older birds.

The development of the microflora is also affected by the digestibility of the diet. Lee (1985) reported that dietary factors like nutrient diversity and nutrient digestibility can influence the ecosystem of the intestinal tract, notably in caeca and large intestine. It has been suggested that the microflora competes with the host animal for dietary nutrients. For a highly digestible diet this competition is usually in favour for the host. However, if birds receive poorly digestible diets rich in non-digestible carbohydrates, more substrate moves to the lower part of the intestinal tract, thus favouring the microflora. The digestive capacity of young animals is still not fully developed (Nitsan *et al.*, 1991; Nir *et al.*, 1993). This leaves more substrate for microbial fermentation.

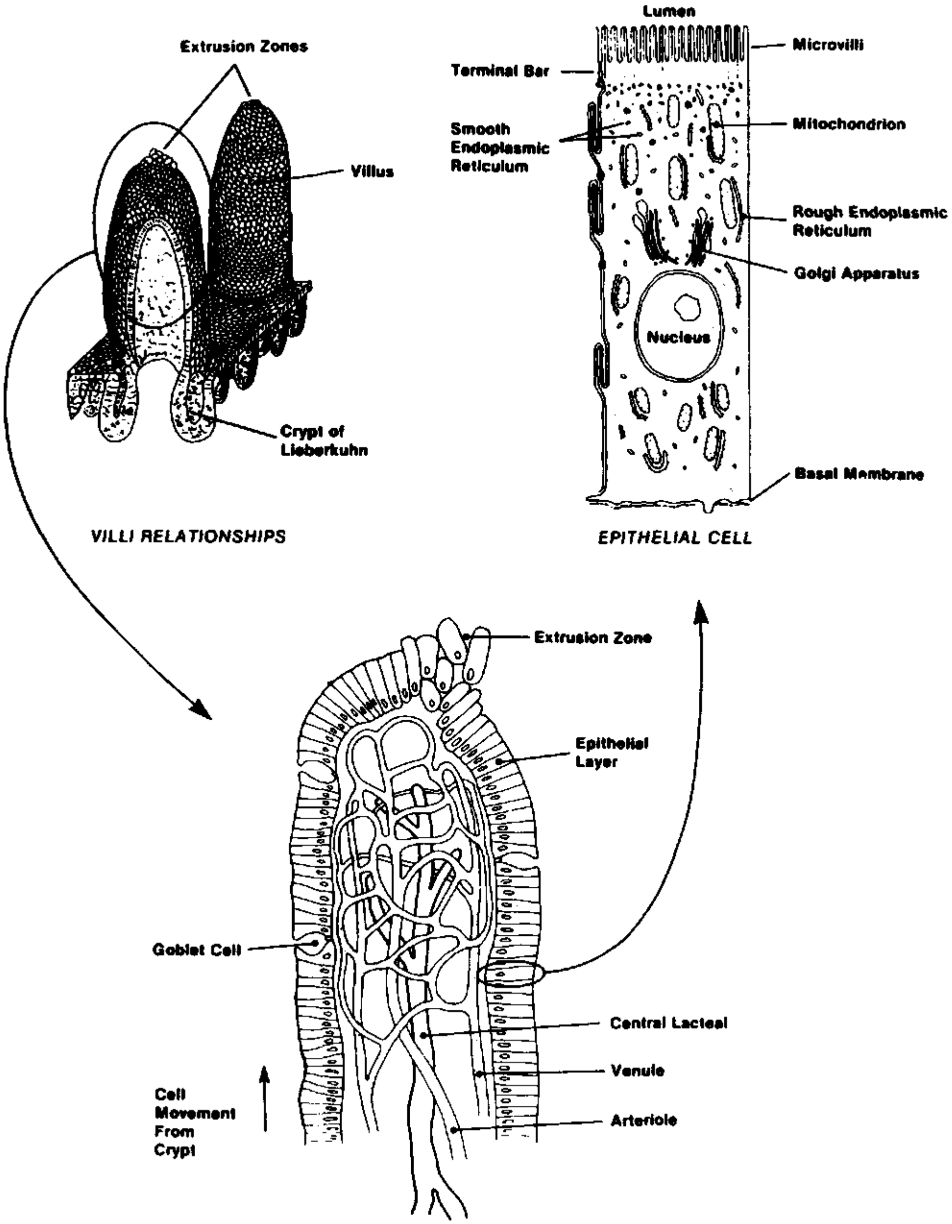
Effect of the microflora on morphology

An increase in bacterial activity in the gastrointestinal tract may affect secretion and morphology of the small intestine (Sakata, 1987). No clear picture of the villi of poultry is found in the literature. Therefore, in Figure 1 a picture of the shape and structure of a villus in pigs is presented. The villi of poultry are similar to those of pigs as far as structure is concerned, but the classification and shape of the villi are different. In poultry, villi appear in a zigzag pattern (Lim and Low, 1977) and are mainly leaf-shaped (Bayer *et al.*, 1975).

The interaction between the microflora and the morphology of the intestinal wall is clearly shown by alterations in the structure and morphology of the gastrointestinal tract of germ-free compared to conventional animals (Heneghan, 1965). The villi in the small intestine of germ-free species are usually uniform in shape and slender, whereas crypts are shorter (Gordon and Bruckner-Kardoss, 1961). In addition, the lamina propria is much thinner in germ-free animals, with a reduction in total number of cells (Abrams *et al.*, 1963). The villus/crypt cell ratio is always higher in germ-free rats, chickens and pigs than in their conventional counterparts, which indicates that less proliferative tissue required to keep germ-free mucosa intact (Heneghan, 1979). The epithelial cell turnover rate in germ-free mice (Abrams *et al.*, 1963) and germ-free chickens (Rolls *et al.*, 1978) was found to be approximately 50% of their respective conventional controls.

Cooke and Bird (1973) associated the presence of pathogenic microorganisms with a change in the intestinal wall and a change in the surface area for nutrient absorption. They demonstrated a shortening of the villi and a decrease in their epithelial layers when counts of pathogenic bacteria increased. In addition, deeper crypts appear. Schneeman (1982) suggests that shorter villi relative to crypt depth result in less absorptive and more secretory cells. This change in morphology in the intestine due to pathogenic organisms is more pronounced in the upper than in the lower parts of the intestinal tract. *Coliform* bacteria (Truscott and Al-Sheikhly, 1977) and *Clostridium* (Kaldhusdal and Hofshagen, 1992) in the intestinal tract causes damage to the mucosal

SWINE INTESTINAL SURFACE



VILLI RELATIONSHIPS

EPITHELIAL CELL

UPPER VILLUS LONGITUDINAL SECTION

Figure 1. *Structure of the intestinal villus and its enterocytes of the pig.*
Source: Moran (1982).

layer of the intestine of broiler chicks. Based on these results it is possible that morphological changes in the intestinal wall are indicative of a disturbance in the balance between non-pathogenic and pathogenic microorganisms. When cell proliferation increases, for example through an increase in microbial activity, the mucin composition of goblet cells may also change (Koninkx *et al.*, 1988). Goblet cells are responsible for the secretion of mucin (Schneeman, 1982). An increase in cell proliferation will reduce the age and maturity of the goblet cells, which might affect the quality of mucin produced by these cells. As a consequence, the absorption of fatty acids may be reduced (Hampson, 1986; Parsons, 1986). In addition, the maturity of enterocytes may also be reduced when cell proliferation increases, which consequently reduces absorption of fatty acids. In addition, a fast turnover of these cells will reduce the age and maturity of the absorptive cells and thus increase the energy requirement for maintenance of the digestive tract and lower the capacity of cells to absorb nutrients.

Effect of the microflora on the health status of the bird

Evidence for the protective role of the indigenous microflora of man and animals against infections of pathogenic microorganisms has been obtained predominantly from studies with either germ-free or antibiotic-treated experimental animals, which are much more susceptible to infections with intestinal pathogens than conventional animals (Hentges, 1980). In addition, the microflora affects the intestinal surface, and hence the receptor places for colonization. Most bacterial species colonize in the lumen (Rosebury, 1962). However, certain microorganisms attach to the intestinal wall. As such the microorganisms can avoid being swept out by the peristaltic flow of the digesta. Some bacteria grow on the surfaces of the villi (Savage, 1972), and others colonize in the crypts of Lieberkuhn and on the epithelium (Savage, 1977; 1983; 1985). It has been suggested that bacteria attach to specific receptors present in the gastrointestinal wall (Ellen and Gibbons, 1972; Barrow *et al.*, 1980; Fuller and Brooker, 1974). Attachment of microorganisms to the intestinal wall takes mostly place by means of fimbriae. The bacterial fimbriae are lectin-like glycoprotein structures that recognize specific oligosaccharides, namely the receptors on the intestinal wall.

Bacteria usually compete for the receptors on the gut wall. This competition between bacteria for receptors is the mechanism by which colonization of pathogens may be prevented by the indigenous flora. Anything that upsets the balance maintained between the host animal and the established microflora may give a pathogen easier access to its target cell and allow it to multiply, thus facilitating competition for target cells (Hirsch, 1980). Therefore, a common approach is to increase the number of desirable bacteria in order to reduce the number of receptors available for colonization of pathogens. This ability of the intestinal microflora to inhibit colonization of invading pathogens is called colonization resistance or competitive exclusion (van der Waaij *et al.*, 1971; Lloyd *et al.*, 1977; Rolfe, 1991).

Colonization resistance against pathogens may be partly result from improvement of the immune system of the bird. Several data in the literature indicate that the microflora affects the immune status of the bird through its influence on the intestinal wall. In the present context, immunity of the animal is the ability of the animal to build up resistance against invasion of pathogenic bacteria. Indications that an interrelationship exists between microbial activity and immunity have been presented by Bienenstock and Befus (1980) who suggest that the immunity of the animal is affected after a change in microbial activity in the intestinal tract. The numbers of lymphocytes, plasma cells and intra-epithelial lymphocytes are lower in germ-free animals than in conventional animals (Crabbe *et al.*, 1970). In addition, Peyer's patches in germ-free animals are smaller and do not show fully developed germinal centres as in their conventional counterparts (Crabbe *et al.*, 1970). Peyer's patches are lymphoid tissues containing all components needed to stimulate an immune response; they are located along the intestinal tract. Pollman *et al.* (1980) have shown that the inclusion of *Lactobacilli* in the diet of gnotobiotic pigs activated the immune system through an increase in the number of leucocytes. Addition of *Lactobacilli* to a diet of pigs (Fuller, 1989) or mice (Perdigon *et al.*, 1987) stimulate the production of antibodies and the activity of phagocytes against pathogenic bacteria in the intestine.

To explain the effects of *Lactobacilli* on the immune system of animals it was suggested that these increase the mucosal immune system (Perdigon *et al.*, 1986, 1987) by increasing IgA secretion into the intestinal lumen, providing adequate defence at the mucosal surface. The presence of antibodies, in particular secretory IgA, is considered to confer a primary line of defence against pathogenic invasions (Fubara and Freter, 1973). Based on this hypothesis several research groups suggest that dietary supplementation of specific live bacteria immunizes these animals against an invasion of pathogenic microorganisms (Morland and Midtvedt, 1984; Perdigon *et al.*, 1986, 1990; Havenaar and Spanhaak, 1994). However, literature data on the effect of *Lactobacilli* on the systemic immune response are scarce.

Carbohydrates and microbial activity

Carbohydrates are the main energy source in poultry diets. The classification of the carbohydrate fraction is complicated due to the great variation in chemical structure and physical properties of carbohydrate sources. In human and animal nutrition carbohydrates are usually classified into sugars, oligosaccharides, starch and non-starch polysaccharides (NSP). The predominant monosaccharides present in poultry diets are glucose and fructose; sucrose is the predominant disaccharide (Carré, 1993). These sugars, as well as starch, are usually well digested by pancreatic enzymes in the small intestine. However, the NSP fractions are not susceptible to the action of pancreatic enzymes (Trowell *et al.*, 1976). These carbohydrates can only be utilized after fermentation by the microbial population, as discussed below.

Mono- and disaccharides

Several studies have been carried out to investigate the nutritive value of pentose sugars, xylose and arabinose (Schutte, 1990; Schutte *et al.*, 1992[a]), galactose and uronic acids (Longstaff *et al.*, 1988). These sugars may be released to some extent from polysaccharides after enzyme supplementation. Replacement of glucose by pentose sugars and uronic acids in broiler diets reduces performance (Schutte, 1990; Schutte *et al.*, 1992[a], Longstaff *et al.*, 1988). Addition of galactose to the diet has little effect on broiler performance (Longstaff *et al.*, 1988). Thus, the energy value of galactose may be similar to that of glucose. This is true only at low dietary inclusion levels (Longstaff *et al.*, 1988). The reduction in performance after inclusion of xylose and arabinose in the diet is partly due to the lower energy value of these monosaccharides compared to glucose (Schutte, 1991). Apparent digestibilities of xylose, arabinose (Schutte, 1990; Schutte *et al.*, 1992[a]) and uronic acids (Longstaff *et al.*, 1988) in poultry are high, ranging between 99% and 65%. However, both research groups showed that the addition of arabinose and, to a lesser extent, xylose and uronic acids to broiler diets increase caecal weights, indicating that these sugars are fermented by microorganisms particularly in the caeca. Addition of xylose (Schutte *et al.*, 1991) or arabinose (Schutte *et al.*, 1992[b]) to pig diets increased the ileal flow of VFA as compared to pigs fed a diet containing glucose. These results suggest that ileal fermentation of xylose and arabinose may occur. Schutte (1991) estimated that, depending on the type of sugar, 10-20% was absorbed by the animal, and 45-80% was degraded by microorganisms.

Mannose is another monosaccharide. *In vitro* studies have shown that mannose inhibits colonization of *Salmonella typhimurium* (Oyofo *et al.*, 1989[c]; Miles, 1993) and *Eschericia coli* (Ofek *et al.*, 1977; Miles ,1993) to cells of the small intestinal wall of the chicken. The same phenomenon has been shown for galactose, which also prevents adherence of *Salmonella typhimurium* to intestinal cells *in vitro* (Oyofo *et al.*, 1989[b]). Addition of 2.5% mannose to the drinking water of broiler chicks has little effect on weight gain, but reduces colonization by *Salmonella typhimurium* (Oyofo *et al.*, 1989[a,b]). Oyofo *et al.* (1989[a,b]) noted that the effect of mannose on *Salmonella* colonization could not be explained by a change in microbial activity because mannose is assumed to be almost resistant to microbial fermentation in the intestinal tract. Colonization of *Enterobacteriaceae* and other bacteria is mediated by a mannose-specific receptor present on the bacterial surface (Freter and Jones, 1976). Therefore, the presence of mannose (depends on the amount) in the intestinal chyme may block the mannose-specific receptors on the surface of pathogenic bacteria, and thus reduce intestinal colonization by these pathogenic bacteria to the epithelial cells.

Several studies have explored the effect of dietary lactose in young broiler chicks on microbial activity. Lactose is a disaccharide present in milk, and can only be digested through microbial degradation, since no lactase is present in the intestinal tract of poultry (Siddons and Coates, 1972). Carré *et al.* (1995[a]) found no clear negative effects on performance of broilers when 3% or 6% lactose was included in the diet. Faecal digestibility values of lactose were, 78% and 64%, respectively.

Addition of lactose to the diet inhibited colonization of *Salmonella* in the caeca of broiler chicks (Oyofo *et al.*, 1989[a,b]; Hinton *et al.*, 1990; Corrier *et al.*, 1991[a,b]). This effect of lactose on *Salmonella* colonization is thought to be due to the action of carbohydrates that alter the microflora by virtue of a specific affinity of bacteria for a specific carbohydrate. Some evidence for discrimination among carbohydrates by the bacteria in the alimentary tract was provided by Lukey (1987) who indicated that bacterial species vary considerably in their capacity to utilize specific carbohydrates. However, little is known about the consequences of these differences on microbial composition in the intestinal tract when specific carbohydrates are added to the diet. Xylose and arabinose are not well utilized by *Lactobacillus acidophilus*, and arabinose not by *Bacteroides fragilis* either (Lukey, 1987). It has been suggested that lactose stimulates the growth of specific bacteria, in particular that of *Lactobacilli* which prefer lactose as a carbohydrate source in contrast to many other bacteria (Morishita *et al.*, 1982; Oyofo *et al.*, 1989[a]). Colonization resistance may explain the effect of lactose on *Salmonella* colonization. On the other hand, addition of lactose to the diet of broiler chicks increases the contents of lactic acid and VFA in the intestine and the caeca, and decreases caecal pH (Corrier *et al.*, 1990; Oyofo *et al.*, 1989[a]; Hinton *et al.*, 1990; Corrier *et al.*, 1991[a]). It is usually accepted that growth of Gram-negative bacteria such as *Salmonella* is inhibited at low pH. Thus, the effect of lactose on *Salmonella* colonization can also be explained by the reduction in pH in the caeca (Corrier *et al.*, 1991[a,b]; Hinton *et al.*, 1991).

Another hypothesis is that lactose enhances the defence mechanism of the bird by affecting the morphology of the intestinal wall. In support of this theory, Tellez *et al.* (1993) have shown that lactose added to the diet reduced the lamina propria thickness of the caeca as well as epithelial cell proliferation. However, it is not known whether the effect of lactose on gut morphology is a direct effect or indirectly mediated by microbial activity.

Oligosaccharides

The main oligosaccharides, named α-galactosides, present in poultry diets are raffinose, stachyose and verbascose. Alpha-galactosides can be degraded by α-galactosidase, which is not secreted into the intestinal tract of monogastric animals (Gitzelmann and Auricchio, 1965). Studies of the effects of oligosaccharides on poultry performance and nutrient digestibility have produced conflicting results. There are indications that α-galactoside fractions in some feedstuffs have anti-nutritional properties in poultry. It has been reported that α-galactosides present in soya bean reduced the true metabolizable energy (TMEn) content in broiler chicks (Coon *et al.*, 1990). In addition, dietary supplementation of 1.4% raffinose reduced the protein efficiency ratio in broiler chickens (Leske *et al.*, 1995). Leske *et al.* (1993) showed that both raffinose and stachyose which are present in soya bean meal reduced the TMEn content. Treviño *et al.* (1990), however, showed that supplementation with α-galactosides isolated from peas in broiler diets had hardly any effect on weight gain and digestion of starch. Brenes *et al.* (1993) concluded that α-galactosides in lupins may be a contributing factor, but not the principal anti-nutritional factor in lupins.

The effects of oligosaccharides on nutrient digestion, absorption and microbial activity may be related to their osmotic properties and fermentation. The presence of non-absorbable water-soluble sugars of low molecular weight in the small intestine, such as α-galactosides, can increase osmolality. The latter may lead to fluid retention in the chyme. Wiggins (1984) suggested an increased rate of passage that could affect absorption of nutrients. Therefore, there may be less time available for fermentation of nutrients by microorganisms. However, α-galactosides are easily fermented by the bacteria in the intestinal tract, which reduce their osmotic effects. This is supported by the finding that the digestibilities of oligosaccharides in poultry vary from 50% to 99% (Brenes *et al.*, 1993; Carré and Lacassagne, 1992; Carré *et al.*, 1995[b]) which stimulate microbial activity (Koopman, 1984). Brenes *et al.* (1989) showed that replacing maize and soya protein isolate by peas in broiler diets hardly affects the composition of the intestinal flora. However, the counts of *Enterococci* in the small intestine and *Clostridia* and *Coliform* bacteria in the caeca of chicks fed peas exceeded those in control chicks.

Several studies have found oligosaccharides linked to fructose, galactose or mannose had positive effects on broiler performance and broiler health. Addition of fructo-oligosaccharides (FOS) to broiler diets improved feed utilization (Ammerman *et al.*, 1988, 1989), whereas other studies showed hardly any effect of FOS or galactose-oligosaccharide on broiler performance (Waldroup *et al.*, 1993; Durst, 1996). The FOS are assumed to be easily fermented by *Lactobacilli* and *Bifidobacteria* (Hidaka *et al.*, 1986; Mitsuoka *et al.*, 1987). *Bifidobacteria*, like *Lactobacilli*, are considered to be beneficial bacteria in the intestinal tract by inhibiting the growth of pathogenic bacteria. Therefore, several research groups have tested the efficacy of dietary FOS against *Salmonella* colonization (Hidaka *et al.*, 1986; Mitsuoka *et al.*, 1987; Baily *et al.*, 1991; Waldroup *et al.*, 1993). These groups have shown that dietary FOS reduce colonization by *Salmonella* in the caeca of broiler chicks. Baily *et al.* (1991), however, found that FOS were not very effective in the prevention of *Salmonella* to colonize in the intestinal tract of poultry and suggested this to be due to fructose which is predominantly fermented in the upper part of the intestinal tract (Table 2).

Table 2. *Effect of 0.75% fructo-oligosaccharide (FOS) in the diet on Salmonella colonization in 7-day-old chicks*[1].

Treatment	Challenge level	*Salmonella*-positive/ chicks tested	CF[2]
Control	10^6	10/21	4.1
Control	10^9	21/22	4.8
+ FOS	10^6	8/22	3.3
+ FOS	10^9	20/23	3.9

[1] Source: Baily *et al.* (1991)

[2] CF (colonization factor) = mean log *Salmonella* count per gram of caecum for all birds within a treatment group.

Sharon (1987) suggests that mannose-oligosaccharides (MOS) are more resistant to bacterial degradation and can, like mannose, bind to the fimbriae of *E. coli*, thus preventing colonization to the intestinal wall. It is, however, questionable whether MOS is resistant to fermentation. More information is needed to confirm this theory.

Water-soluble viscous NSP

It is well established that the NSP fractions present in barley, rye and wheat have anti-nutritive properties in poultry. It is assumed that the anti-nutritive effects of barley are associated with the water-soluble fraction of β-glucans (White *et al.*, 1981; 1983; Fincher and Stone, 1986; Classen *et al.*, 1985, 1988; Campbell *et al.*, 1989) and those of rye (Antoniou and Marquardt, 1981; Antoniou *et al.*, 1981; Ward and Marquardt, 1987) and wheat (Choct and Annison, 1990, 1992[a,b]) to the water-soluble fraction of arabinoxylans (pentosans). The anti-nutritional effects of these water-soluble NSP fractions, which are manifested by growth depression, reduced feed utilization and wet droppings, which are more pronounced in young than in older birds (Viveros *et al.*, 1994; Ward and Marquardt, 1983; Veldman and Vahl, 1994). The reduced performance is a result of a decrease in nutrient digestibility, in particular that of fat (Antoniou *et al.*, 1981; Campbell *et al.*, 1983; Choct and Annison, 1992[a]). It is well documented that supplementation of an enzyme preparation to barley-based diets (Hesselman *et al.*, 1981; Hesselman and Åman, 1986; Schutte *et al.*, 1993[a]), rye-based diets (GrootWassink *et al.*, 1989; Classen and Bedford, 1991; Bedford *et al.*, 1991; Bedford and Classen, 1993) and wheat-based diets (Annison, 1992; Schutte *et al.*, 1993[b]) can eliminate (completely or partly) the anti-nutritive properties of β-glucans and pentosans in chicks.

Another NSP fraction is pectin, which is present in all higher-plant tissues (e.g. soya bean meal, rapeseed meal and sunflower meal). Pectins have a great variability in chemical structure and water solubility (May, 1990). Pectin is characterized by a linear backbone of 1→4-linked α-D-galacturonic acid units. The carboxyl groups of D-galacturonic acid are methylated to a different extent. Knowledge of the effects of pectin on broiler performance is mainly based on studies with highly methylated citrus pectin. Results of these studies showed a clear depressing effect on growth rate and feed utilization after addition of citrus pectin to broiler diets (Vohra and Kratzer, 1964; Wagner and Thomas, 1977; Patel *et al.*, 1980; 1981; Bishawi and McGinnis, 1984).

The mechanism by which water-soluble carbohydrates exert their anti-nutritional effects is supposed to be related to their viscous properties contributing to a higher viscosity of the aqueous fraction of the intestinal digesta. This is well documented for β-glucans (White et al, 1981; Wang *et al.*, 1992; Salih *et al.*, 1991) and pentosans (Ward and Marquardt, 1987; Fengler and Marquardt, 1988; Choct and Annison, 1992^b) included in the diets of broiler chicks. This increase in intestinal viscosity has been suggested to alter several physiological mechanisms in the bird. Edwards *et al.* (1988) demonstrated *in vitro* that the transport of glucose and sodium towards the epithelial cells was impaired in a viscous environment. Moreover, the increase in intestinal viscosity may reduce diffusion of pancreatic enzymes to dietary nutrients and impair transport to the epithelial surface. This may limit hydrolysis of nutrients by digestive enzymes, which consequently decreases nutrient digestion (Antoniou *et al.*, 1980; Fengler and Marquardt, 1988). Ikegami *et al.* (1990) and Poksay and Schneeman (1983) have shown that the inclusion of various viscous polysaccharides, such as pectin and guar gum, in diets of rats increases the activity of enzymes in the gastrointestinal tract. In contrast, Larsen *et al.* (1993) report that enzyme activities are hardly affected when rat diets are supplemented with viscous carboxymethyl cellulose (CMC). This indicates that viscosity *per se* does not affect pancreatic-biliary secretions.

Nutrients have to pass the epithelial cells for absorption take place. Another hypothesis to explain the anti-nutritive effects of the water-soluble NSP is that this NSP fraction interacts with the mucus and/or glycocalyx by slowing the rate of absorption. Addition of gelling agents such as gum and pectin to the diet of rats increased the thickness of the unstirred water layer (Johnson and Gee, 1981; Johnson *et al.*, 1984) and decreased the activity of specific epithelial surface enzymes (Johnson and Gee, 1986). Viveros *et al.* (1994) noted a change in the morphology of the intestinal wall of the jejunum when birds were fed a barley-based compared to a maize-based diet and found shorter and thicker villi and an increase in number of goblet cells in birds fed the barley-based diet. The addition of a β-glucanase enzyme preparation could counteract these effects. Smits (1996), however, showed no effect of addition of CMC to broiler diets on the morphology of the intestinal wall. This indicates that digesta viscosity *per se* does not affect gut morphology. Jacobs (1983) showed that the mucosal structure of the small intestinal of rats is affected by dietary fibre, and that these changes depend on the fibre source present. Gee *et al.* (1996) concluded that the effect of gelling agents on mucosal conditions depends on their fermentability. There is no information available on whether the effect of water-soluble NSP fractions on gut morphology is a direct effect or is mediated by the gut microflora.

Satchithanandam *et al.* (1990) found indications that dietary fibre may increase the secretion of mucus in digesta. This may explain the high endogenous losses in poultry when water-soluble NSP are included in broiler diets. This notion is supported by findings of Angkanapom *et al.* (1994) who showed an increase in endogenous protein losses when wheat pentosans were added to a broiler diet. This effect of water-soluble NSP on endogenous losses may also be due to an increase in biomass in excreta.

Interaction between water-soluble viscous carbohydrates and the microflora

Wagner and Thomas (1978) showed an increase in counts of anaerobic bacteria in the small intestine when birds were fed a rye-based diet or a diet containing citrus pectin compared to those fed a maize-based diet (Table 3). These investigators attributed the anti-nutritive activity of rye and citrus pectin to an increase in bacterial activity, which they associated with that of spore-forming bacteria. Several studies support the theory that the microflora may play a role in the impact of the anti-nutritional effect of viscous water-soluble NSP. Supplementation of penicillin to a rye-based diet markedly improved performance of birds (Marquardt *et al.*, 1979). Marquardt *et al.* (1979) suggested that penicillin suppresses the microflora which is enhanced by the anti-nutritive effects of rye. In addition, Antoniou and Marquardt (1982) reported that the magnitude of the effect of penicillin was greater in rye-based diets containing 8% tallow than in diets with 3% tallow. Campbell *et al.* (1983) showed that the depression in weight gain and in fat retention in rye-fed birds was more pronounced in conventional chicks than in germ-free chicks. Administration of *Streptococci* to germ-free chicks induced the type of response found in conventional chicks. Smits and Annison (1996) noted that the anti-nutritive property of viscous CMC on fat digestibility was negligible in germ-free chicks, although ileal viscosity was markedly increased. Smits and Annison (1996) found the anti-nutritional properties of CMC to be reduced in the absence of microflora. Vukic Vranjes and Wenk (1995) showed that addition of an antibiotic and a β-glucanase enzyme preparation to a barley-based diet increased fat digestibility in broilers. Thus, the positive effect of both feed additives, antibiotics and enzymes, is probably related to their interaction with the microflora.

Hofshagen and Kaldhusdal (1992) reported that the inclusion of barley to a wheat- and oat-based diet increased the number of *Clostridia* in the small intestine of broilers. Based on these results, it was suggested that viscous water-soluble NSP increase microbial activity, in particular that of *Clostridia* and *Enterobacteriaceae*. In *in vitro* studies, however, the water-soluble fraction from wheat inhibited the growth of *Clostridium perfringens* (Branton *et al.*, 1996). This indicates that the carbohydrate fraction *per se* does not cause the change in the microbial population. Possibly, the elevated level of undigested nutrients in the lower part of the small intestine, which is associated with increased digesta viscosity, promotes microbial growth. This leads to

Table 3. *Effect of dietary rye and pectin on total anaerobic colony forming counts in the ileum of broiler chicks* [1,2].

Experiment	Birds fed the experimental diets for 4 days			Birds fed the experimental diets for 14 days		
	maize diet	rye diet	maize diet + 4.5% pectin	maize diet	rye diet	maize diet + 4.5% pectin
1	9.3	920	130	2.9	480	690
2	6.9	1280	430	0.2	90	150
3	0.5	50	3400	11.8	660	510
4	4.3	750	450	1.4	1900	2000
mean	4.3	750	1102	4.1	782	837

1) Source: Wagner and Thomas (1978).

2) Counts per cm of ileum (x 10^7). Each observation represents a mean of duplicate cultures from a composite sample of ileum of five chicks.

the assumption that the microbial population in the small intestine in particular is affected by water-soluble viscous NSP. This is supported by Choct *et al.* (1996) who showed that viscous NSP from wheat increased ileal viscosity and VFA concentration, whereas the VFA concentration in the caeca was hardly affected.

Caecal VFA concentration, however, was markedly elevated by addition of an enzyme to the diet containing the NSP fraction from wheat, whereas ileal viscosity and VFA concentration were reduced. Carré and Gomez (1994) showed that addition of a pectin-based gelling agent to the diet of either intact or caecectomized adult cockerels increased the production of lactic acid and VFA in the ileal digesta. The effect on the production of lactic acid and VFA was more pronounced in intact than in caecectomized cockerels. Those results are in agreement with findings of Choct *et al.* (1992) who showed that the anti-nutritive effect of wheat pentosans was more pronounced in intact than in caecectomized broilers. Thus, also the caeca can play a role in the anti-nutritive effects of water-soluble NSP. It may be speculated that several bacteria of the caeca migrate towards the small intestine when the ileal viscosity is increased.

Goodlad *et al.* (1989) suggested that the microflora plays an important role in the effects of dietary fibre on mucosal conditions. They found no mucosal hyperplasia in germ-free rats when fibre was included in the diet. Wyatt *et al.* (1988) suggested that an increase in bulk in the small intestine, irrespective of the nature of the bulk, causes mucosal hypoplasic effects of non-digestible polysaccharides. Salih *et al.* (1991) showed that the passage rate was reduced in birds fed hullness barley in the diet compared to birds fed a maize-based diet. Addition of the viscous CMC to the diet increased ileal viscosity and increased retention time in broiler chicks (van der Klis and van Voorst, 1993). It has been hypothesized that when the digesta stays in the intestinal tract for a longer time, more time is available for fermentation of the nutrients by microorganisms in the small intestine.

Insoluble NSP

Insoluble NSP are not degraded to a large extent by bacterial fermentation in poultry. In addition, there are no indications that insoluble NSP interferes with nutrient digestibility (Akiba and Matsumoto, 1980). Literature data, however, indicate that dietary insoluble NSP may modify the anti-nutritional activity of water-soluble NSP. Mollah and Annison (1981) and Rogel *et al.* (1987) reported that fibrous materials such as oat hulls improve starch digestibility of wheat low in ME. Insoluble NSP have water-holding properties and increase bulkiness (Cummings, 1981, Robertson and Eastwood, 1981). In addition, it was shown that insoluble carbohydrates increase the passage rate in rats

(Robertson, 1988; Eastwood, 1992). Particle size and structure are related to these effects, at least in part (Robertson, 1988). Therefore, it can be argued that the effect of insoluble NSP on the anti-nutritional effect of viscous water-soluble NSP is related to the laxative properties of these carbohydrate fractions. Furthermore, it has been speculated that microorganisms adhere to insoluble NSP (Rogel *et al.*, 1987; Smits and Annison, 1996).

Effect of gut microflora and carbohydrates on fat digestion

It has been estimated that up to 10^{14} bacteria can be found in the lumen of the digestive tract of broiler chicks (Mead, 1989). Thus, bacteria in the digestive tract can play an important role in metabolism in the intestinal tract (Savage, 1986; Fuller and Cole, 1988). However, knowledge of the significance of the intestinal microflora to the nutrition of the chicks is scarce. Utilization of dietary energy after microbial degradation of nutrients and energy is less than after enzymatic hydrolysis and digestion of nutrients (Black, 1995). In pigs these losses are ranged between 33% (ARC, 1981) and 50% (Just *et al.*, 1983; van Es, 1974). No published data for poultry are available. Several studies have shown that chickens are able to utilize the acids synthesized by bacteria as energy source. The efficiency of utilization, however, is low (Hedge *et al.*, 1982; Muramatsu *et al.*, 1991). This energy is mainly derived from lactic acid and VFA.

Several studies indicate that N utilization, too, is decreased in conventional chicks compared to germ-free chicks (Furuse and Yokota, 1984; 1985). Part of this reduction in N can be explained by the fact that the microorganisms can incorporate dietary amino acids into microbial protein (Salter and Coates, 1974). There is also increasing evidence from work with rats that an increase in microbial activity stimulates proliferation of mucosal cells (Sakata, 1987; Goodlad *et al.*, 1989). This proliferation is probably associated with increased losses of epithelial cells, which increases endogenous losses. This contributes to a greater faecal N output and, therefore, to a decrease in the apparent digestibility of N.

Germ-free chicks perform better than conventional chicks (Forbes and Park, 1959; Coates *et al.*, 1963). This implies that the detrimental effects of the microflora are more pronounced than their possible beneficial effects (Coates, 1976; Ratcliffe, 1985). Although it should be noted that a microflora is necessary to prevent colonization of pathogenic bacteria. Beneficial and negative effects of the microflora are summarized in Table 4.

Table 4. *The effects of gut microorganisms on metabolism*

Beneficial effects	Negative effects
- Synthesis of B-vitamins and vitamin K	- Release of hepatotoxic substances such as endotoxins (lipopolysaccharides, LPS)
- Detoxification of food components or endogenous products	- Release of toxins from non-toxic precursors
- Recovery of endogenous nitrogen	- Incorporation of nutrients, e.g. amino acids in microbial mass
- Production of digestive enzymes, e.g. bacterial amylase for starch digestion	- Modification of nutrients
- Synthesis of organic acids	- Decreased digestibility of fat due to altered lipids and bile acids

Adapted from Coates (1980), Rowland and Walker (1983), Nostrandt (1990), Muramatsu *et al.* (1991), Pappo *et al.* (1992).

Fat digestion

It is well documented that fat digestibility is affected in particular by the intestinal microflora. Fat absorption consists of three sequential steps (see Figure 2). Firstly, the large fat droplets in the diet are mechanically dispersed to finely emulsified particles. Next, the lipid esters (triglycerides) are enzymatically hydrolysed by pancreatic lipase to free fatty acids and glycerol. Hydrolysis takes place at the oil-water interface. Before absorption, the fatty acids have to form micelles. Bile acids are necessary to form the emulsified particles and micelles. Bile acids, cholic acid and deoxycholic acid are conjugated to glycine or taurine. The secretion of lipase could be a limiting factor in digestion of fat in young birds up to two weeks of age (Nitsan *et al.*, 1991; Nir *et al.*, 1993). This notion is supported by the finding that addition of a lipase to the diet improved fat digestion in chicks up to three weeks of age (Polin *et al.*, 1980). Young birds also have a limited capacity to produce bile salts (Green and Kellog, 1987; Iñarrea *et al.*, 1989). This corresponds with the finding that the digestibility of fat is lower in young broiler chicks than in older birds (Ketels and de Groote, 1988). Addition of bile acids to broiler diets improves fat digestion (Gomez and Polin, 1974; 1976; Polin *et al.*, 1980; Kussaibati *et al.*, 1982[a]). Kussaibati *et al.* (1982[b]) noted that the effect of dietary bile acids on fat digestibility was more pronounced in conventional chicks than in germ-free chicks. This indicates that sufficient amounts of bile acids in the gastrointestinal tract may be associated, at least in part, with the presence of microflora.

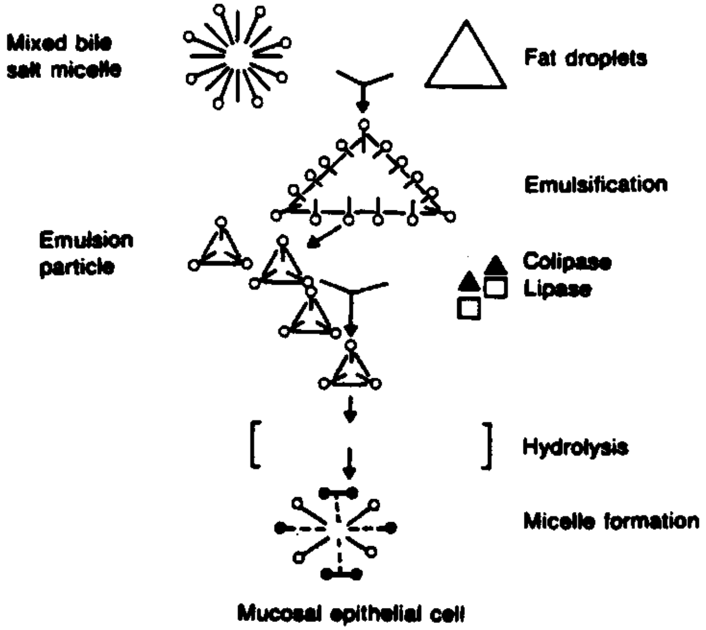


Figure 2. *The digestion and absorption of dietary fat.*
Adapted from Stremmel (1987).

Poultry can efficiently recycle bile acids through reabsorption of bile acids from the intestinal tract, which is the enterohepathic cycle of these acids (Hulan and Bird, 1972; Juste *et al.*, 1979). In young birds, a reduced recycling of bile acids causes a decrease in the bile acid pool size, reducing the bile acid concentration in the intestinal digesta (Juste *et al.*, 1983). If the concentration is lower than the so-called critical micellar concentration, mixed micelles formed by bile salts spontaneously dissociate (Freeman, 1969). As a result, fat emulsification and formation of micelles is less, and fat digestion is reduced. In this respect, the digestion of the long chain and saturated fatty acids would be more sensitive to any inadequacy than the soluble short chain and long chain unsaturated fatty acids. Westergaard and Dietschy (1976) and Brink *et al.* (1995) found the polarity and solubility of saturated long chain fatty acids to be more resistant to their movement in the intestine than that of soluble short chain and long chain unsaturated fatty acids. Krogdahl (1985) proposed that the hydrophobic core of the micelles formed by the interaction of bile acids and short chain and long chain unsaturated fatty acids is able to solubilise long chain saturated fatty acids. Both adequate concentrations of short chain and long chain unsaturated fatty acids and bile acids are required for the maximum absorption of long chain saturated fatty acids (Garrett and Young, 1975).

The mucus layer is an important barrier for absorption of nutrients, in particular that of fatty acids (Smithson *et al.*, 1981; Wilson and Dietschy, 1974). Fatty acids are absorbed at the top of the villi (Bézard and Bugaut, 1986; Caspary, 1992). The capacity of the bird to absorb fatty acids depends on several factors. An important factor is the quality of the mucin produced by goblet cells (Wilson and Dietschy, 1974; Smithson *et al.*, 1981). Because an increased microbial activity in the small intestine affects the mucosal layer, absorption of fatty acids may also be reduced due to morphological changes.

Microflora and fat digestion

Bacteria can degrade bile acids through deconjugation, dehydration and dehydrogenation. Hydrolysis of bile acids changes their efficacy to solubilize fat (Hofmann and Mysels, 1992). Of these different transformation forms of bile acids, the most common reaction is deconjugation. During deconjugation the acid-amide binding is hydrolysed by either cholylglycine hydrolase or cholyltaurine hydrolase. Many intestinal bacteria, including *Streptococcus*, *Enterococcus*, *Bifidobacterium*, *Clostridium*, *Bacteroides* and *Lactobacillus*, are able to catalyze deconjugation of bile acids *in vitro* (Coates, *et al.*, 1981; Masuda, 1981; Hylemond, 1985; Chateau *et al.*, 1994; Grill *et al.*, 1995). Cole and Fuller (1984) indicate that not every bacterial species has the same activity in catalyzing the deconjugation of bile acids. However, there is no information available on this activity of bacterial species *in vivo*. Bile salt hydrolase activity has been partially purified only from *Clostridium perfringens* (Nair *et al.*, 1967) and *Bacteroides fragilis* (Aries and Hill, 1970).

Water-soluble NSP and fat digestion

Water-soluble NSP decrease fat digestion in particular (Antoniou *et al.*, 1981; Campbell *et al.*, 1983; Choct and Annison, 1992^a, Smits, 1996). Antoniou *et al.* (1980) and Antoniou and Marquardt (1982) found an interaction between fat source and the anti-nutritive effects of rye on broiler performance. They found a greater decrease in fat digestibility in rye-fed chicks when tallow instead of soya oil was included in the diet. Ward and Marquardt (1983) attributed this effect to the degree of saturation and chain length of the fatty acids in these fats. It has been suggested that viscous NSP reduce the availability of bile acids for emulsification of fats, reducing fat absorption. This is supported by findings of Campbell *et al.* (1983) and Fengler *et al.* (1988), who showed that addition of conjugated bile acids to rye-based diets improves animal fat digestibility. In addition, supplementation of viscous CMC to the diet of broiler chicks increased secretion of bile acids in the excreta, and reduced bile acid concentrations in the small intestine (Smits, 1996). It seems likely that the effect of water-soluble NSP is mediated by deconjugation of bile acids due to an increase in microbial activity. This theory is supported by Feighner and Dashkevicz (1988) who showed that a rye-based diet had a higher cholyltaurine hydrolase level than a maize-based diet. In that study also a sucrose-containing diet was used. Sucrose had no effect on the content of cholyltaurine hydrolase relative to that found in birds on the maize-based diet. Thus, NSP may increase hydrolase activity in the intestinal tract and consequently increase the deconjugation rate.

Deconjugated bile acids can be readily absorbed in the small intestine of man (Setchell *et al.*, 1982). However, the reutilization of deconjugated bile acids from portal blood is less effective than that of conjugated bile acids. Studies in man have shown that only 40-50% of deconjugated bile acids is recycled (Angelin *et al.*, 1982). An increase in bile acid deconjugation may, therefore, reduce the bile acid pool size, and consequently the concentration of bile acid, particularly in young birds. Another hypothesis is that bile acid deconjugation limits the potential for formation of micelles. This will reduce fat digestion, particularly digestion of fats containing long-chain saturated fatty acids.

The role of feed additives

Based on the above reasoning, it can be concluded that the composition of the diet can easily disturb the balance in the gut ecosystem of young broiler chicks. As a consequence, the health status of the birds may be more sensitive to disturbances. Moreover, nutrient utilization is reduced. To counteract these problems, feed additives that can alter gut microflora are normally included in the diet.

Growth-promoting antibiotics

Broiler diets are normally supplemented with growth-promoting antibiotics. It is well recognized that dosages of antibiotics far below the 'therapeutic' range enhance performance, in particular by increasing the utilization of dietary energy (Eyssen and DeSomer, 1963; Nelson *et al.*, 1963; March *et al.*, 1978). The primary action of growth-promoting antibiotics is to reduce the counts of Gram-positive bacteria (MacKinnon, 1985). However, quantitative changes in populations of intestinal microbial species have not been observed consistently. Evidence that the effect of antibiotics is through their action on the intestinal microflora is provided by Coates *et al.* (1963) and Freeman *et al.* (1975) who have shown that inclusion of antibiotics in broiler diets under germ-free conditions has hardly any effect on performance. The exact mode of action of growth-promoting antibiotics, however, is still not fully understood. Several mechanisms have been proposed to explain the effects of growth-promoting antibiotics (Visek, 1978; Armstrong, 1986). Several mechanisms are frequently mentioned:

1) Microbial activity is reduced, and thus the direct competition between the bacteria and the host for nutrients and energy is more in favour of the host animal.
2) Production of toxic metabolites by microbes (e.g. amine, ammonia) is reduced. These are known to damage the gut epithelium and to hinder nutrient absorption.
3) There is enhanced efficiency of absorption and utilization of nutrients because the wall of the intestinal tract is thinner.
4) Microorganisms responsible for subclinical infections are suppressed.
5) Reduction in the attachment of pathogens to uncovered/damaged epithelial cells, which makes them less susceptible to endotoxins.

There is, however, an increasing concern regarding the possible emergence of antibiotic-resistant strains of species pathogenic to man, resulting in public and political rejection of the use of growth-promoting antibiotics in animal diets, and stimulating the demand for alternative feed additives which are more 'natural' in concept.

Pre- and probiotics

Poultry products have been incriminated in the transmission of *Salmonella* spp. and *Campylobacter* spp. in cases of human disease. Therefore, much interest has been generated towards alternative feed additives to manipulate the intestinal flora in order to reduce colonization by these pathogens. Data on the efficacy of alternative feed additives on broiler performance and subsequent nutrient utilization are scanty.

In order to prevent colonization of pathogens in the intestinal tract several approaches have been studied with varying degrees of success. One approach is to incorporate fermentable sugars, so-called prebiotics, in the diet or the drinking water. The main objective of prebiotics is to promote the growth of specific beneficial bacterial strains such as *Bifidobacteria* or *Lactobacilli*, or to reconstitute the microbial ecosystem to its normal composition. Most literature data about prebiotics relate to lactose and oligosaccharides linked to specific sugars.

Another approach to manipulating the intestinal microflora is by application of (viable) bacterial preparations, so-called probiotics. The concept of probiotics is based on competitive exclusion, the process by which colonization by pathogens can be prevented by increasing the number of beneficial bacteria. Two types of probiotics are discussed in the literature, namely the direct-fed microbials (DFM) and the undefined or defined bacterial cultures obtained from pathogen-free adult chicken caecal or faecal matter. DFM are single or a limited number of well defined bacterial strains. The lactic acid-producing bacteria *Lactobacillus* and *Bifidobacterium* have been primarily used for this purpose. But also other strains, such as *Bacillus* and *Aspergillus,* are used. The bacterial cultures, the so-called competitive exclusion (CE) cultures, are based on the work of Nurmi and Rantala (1973) who were the first to demonstrate that administration of a gastrointestinal flora from healthy adult birds to newly hatched chicks reduces the prevalence of enteropathogens, especially *Salmonella.* The specific bacteria responsible for this effect have not been identified conclusively. The presence of viable anaerobic organisms is essential (Rantala, 1974). Later, numerous attempts have been made to find a combination of bacteria with protective properties. These defined cultures, however, were less effective than the undefined cultures.

Concerning the mode of action of probiotics, several hypothesis have been postulated (Pollman, 1986; Fuller, 1989), but still many speculations exist. The most frequently suggested mechanisms are the suppression of viable pathogens by competition for adhesion to cells, stimulation of the immune system and production of anti-bacterial compounds. The colonization of bacteria in the intestinal mucosa as well as the effect of bacteria on the immune system were discussed above.

The effects documented in the literature with regard to the reduction in colonization of pathogens through addition of pre- and probiotics to the diet are rather consistent. Thus, the concepts of prebiotics and probiotics are valid. However, the results with regard to the effects of pre- and probiotics on broiler performance and nutrient utilization are variable and unpredictable (Gorbach, 1990). This is due in particular to the fact that many factors are involved in regulation of the balance of the gastrointestinal microflora. The effect of these factors on the efficacy of pre- and probiotics, however, is still unknown. One of the factors that may interfere with the effects of pre- and probiotics is the composition of the diet. The composition of the diet can affect the balance in the gut ecosystem. Therefore, it may be hypothesized that an interaction exists between the efficacy of pre- and probiotics and other dietary constituents. Another dietary factor that may interfere with the efficacy of pre- and probiotics are feed additives other than growth-promoting antibiotics. In broiler diets normally anticoccidials are included. Anticoccidials also have bacteriostatic properties, and consequently may interfere with the effect of pre- and probiotics. Therefore, information is needed about the effect of pre- and probiotics on nutrient utilization and colonization by pathogens in relation to diet composition.

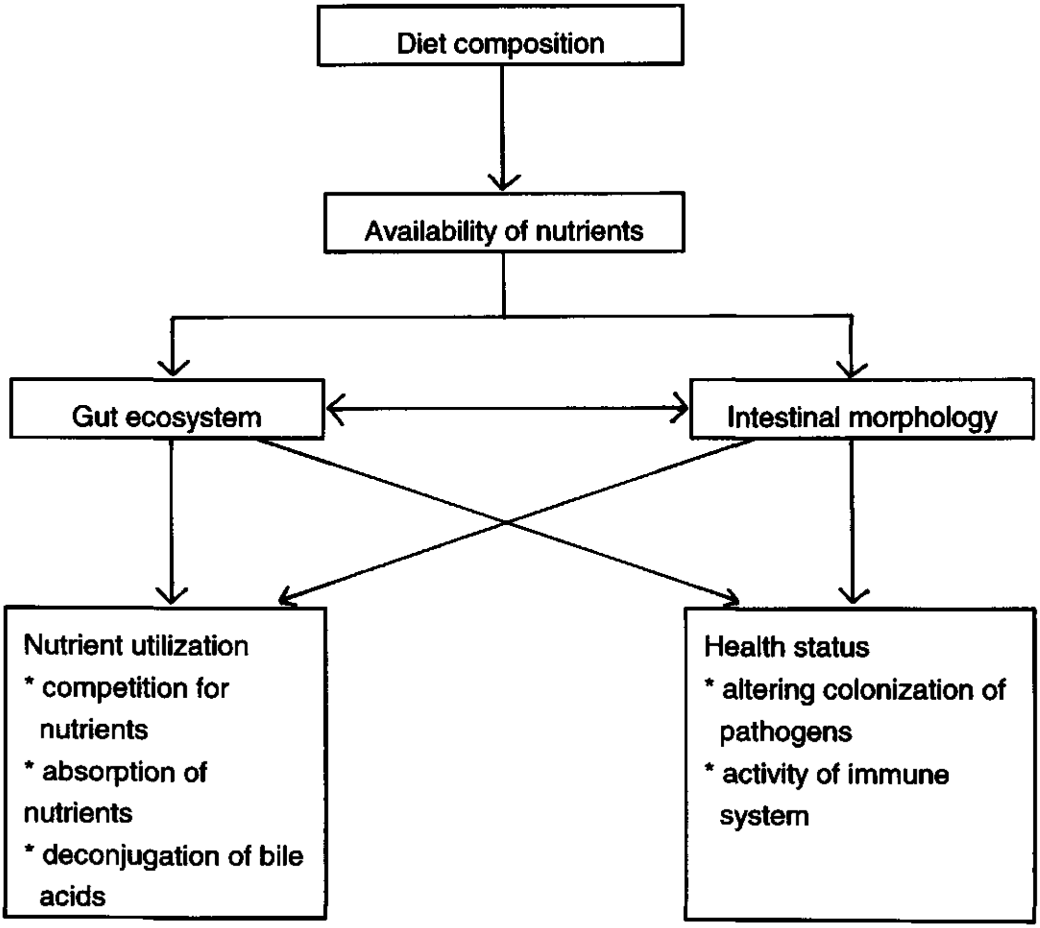


Figure 3. *The role of the intestinal microflora on nutrient utilization and health status of broiler chicks.*

Concluding remarks

The gut microflora is a complex ecosystem, which can be readily disturbed in young broiler chicks. From this review it can be concluded that the gut microflora plays an important role in the health status of the bird and in the digestion and absorption of nutrients (Figure 3). In particular, the composition of the microflora may be involved in the health status of the bird. Specific bacterial strains are thought to prevent colonization by pathogens and to improve the (local) immune system. The effect of gut microflora on the digestion and absorption of nutrients appears to be mainly related to the activity of the microflora in the intestinal tract. Particularly, an increase in microbial activity in the small intestine may reduce the hydrolysis and absorption of nutrients. Literature data indicate that an increase in amount of fermentable substrate in the lower part of the small intestine, also in relation to the passage rate of the chyme, increases microbial activity.

As yet, it can be concluded that broiler diets should aim at minimizing the microbial activity in the small intestine and reducing growth of undesirable bacteria (*E. coli*, *Clostridia*, etc.) in favour of beneficial bacteria (*Lactobacilli*, *Bifidobacteria*). Further research is required on the interrelationships between dietary factors and the composition and activity of the gut microflora. In addition, the role of bacterial species on nutrient digestibility and on the health status of the bird has to be defined. Therefore, these gaps in our knowledge have to be filled before diets can be optimized to achieve an optimal 'balance' in the microflora.

References

Abrams, G.D., Bauer, H. & Sprintz, H. (1963) Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. *Laboratory Investigations* 12: 355-364.

Agricultural Research Council (1981) The nutrient requirement of pigs. Slough: Commonwealth Agricultural Bureaux.

Akiba, Y. & Matsumoto, T. (1980) Effects of several types of dietary fibres on lipid content in liver and plasma, nutrient retentions and plasma transaminase activities in force-fed growing chicks. *Journal of Nutrition* 110: 1112-1121.

Ammerman, E., Quarles, C. & Twinning, P.V. Jr. (1988) Broiler response to the addition of dietary fructooligoasaccharides. *Poultry Science* 67 (Suppl. 1): 46(Abstracts).

Ammerman, E., Quarles, C. & Twinning, P.V. Jr. (1989) Evaluation of fructooligoasaccharides on performance and carcass yield of male broilers. *Poultry Science* 68 (Suppl. 1): 167 (Abstracts).

Angelin, B., Bjorkhem, I., Einarsson, K. & Ewerth, S. (1982) Hepatic uptake of bile acids in man. *Journal* *of Clinical Investments* 70: 724-731.

Angkanaporn, K., Choct, M., Bryden, W.L. & Annison, E.F. (1994) Effects of wheat pentosans on endogenous amino acid losses in chickens. *Journal of the Science of Food and Agriculture* 66: 399-404.

Annison, G. (1992) Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology* 38: 105-121.

Antoniou, T. & Marquardt, R.R. (1981) Influence of rye pentosans on the growth of chick. *Poultry Science* 60: 1898-1904.

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

Antoniou, T., Marquardt, R.R. & Misir, R. (1980) The utilization of rye by growing chicks as influenced by calcium, vitamin D_3 and fat type and level. *Poultry Science* 59: 758-769.

Antoniou, T., Marquardt, R.R. & Cansfield, E. (1981) Isolation, partial characterization and anti-nutritional activity of a factor (pentosans) in rye grain. *Journal of Agriculture and Food Chemistry* 29: 1240-1247.

Aries, V. & Hill, M.J. (1970) Degradation of steroids by intestinal bacteria. I. Deconjugation of bile salts. *Biochemistry Biophysica Acta* 202: 526-534.

Armstrong, E.G. (1986) Control and Manipulation of animal growth (Butery, P.J., Lindsay, D.B. & Hanyes, N.B., eds.) Butterworths, London, pp. 21-37.

Bailey, J.S., Blankenship, L.C. & Cox, N.A. (1991) Effect of fructooligoasaccharides on *Salmonella* contamination of the chicken intestine. *Poultry Science* 70: 2433-2438.

Barnes, E.M., Mead, G.C., Barnum, D.A. & Harry, E.G. (1972) The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Competitive exclusion of salmonellas from the newly hatched chick. *British Poultry Science* 12: 311-326.

Barrow, P.A., Brooker, B.E., Fuller, R. & Newport, M.J. (1980) The attachment of bacteria to the gastric epithelium of the pig and its importance in the micro-ecology of the intestine. *Journal of Applied Bacteriology* 48: 147-154.

Bayer, R.C., Chawan, C.B., Bird, F.H. & Musgrove, S.D. (1975) Characteristics of the absorptive surface of the small intestine of the chicken from 1 day to 14 weeks of age. *Poultry Science* 54: 155-162.

Bayer, R.C., Hoover, W.H. & Muir, F.V. (1978) Dietary fiber and meal feeding influence on broiler growth and crop fermentation. *Poultry Science* 57: 1456-1459.

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

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

Bezard, J. & Buguat, M. (1986) Absorption of glycerides containing short, medium and long chain fatty acids. In: *Fat absorption, Volume I.* (Kuksis, A., ed.), CRC Press Inc., Boca Raton, Florida, pp. 119-158.

Bienenstock, J. & Befus, A.D. (1980) Mucosal immunity. *Immunology* 42: 249-270.

Bishawi, K.O. & McGinnis, J. (1984) Studies on the action of pectin in depressing the growth of chicks. *British Poultry Science* 25: 519-528.

Black, J.L. (1995) Modelling energy metabolism in the pig - critical evaluation of a simple reference model. In: *Modelling growth in the pig.* (Moughan, P.J., Verstegen, M.W.A. & Visser-Reyneveld, M.I., eds.) EAAP Publication no. 78, Wageningen Press, Wageningen,The Netherlands, pp. 87-103.

Branton, S.L., Latt, B.D., May, J.D., Hedin, P.A., Austin, F.W., Latour, M.A. & Days, E.J. (1996) The effects on nonautoclaved and autoclaved water-soluble sheat extracts on the growth of *Clostridium perfringers. Poultry Science* 75: 335-338.

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

Brenes, A., Treviño, J., Centeno, C. & Yuste, P. (1989) Influence of peas (*Pisum Savitum*) as a dietary ingredient and flavomycin supplementation on the performance and intestinal microflora of broiler chicks. *British Poultry Science* 30: 81-89.

Brink, E.J., Haddeman, E., De Fouw, N.J. & Westrate, J.A. (1995) Positional distribution of stearic and oleic acid in a triacylglycerol and dietary calcium concentration determines the apparent absorption of these fatty acids in rats. *Journal of Nutrition* 125: 2379-2387.

Brown, R.H. (1996) The lliving gut must be considered when trying to eliminate pahtogens. *Feedstuffs* 68: 14.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens; effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. *British Poultry Science* 24: 191-203.

Campbell, G.L., Rossnagel, B.F., Classen, H.L. & Thacker, P.A. (1989) Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. *Animal Feed Science and Technology* 26: 221-230.

Carré, B. (1993) Digestibility of carbohydrates in poultry. In: *Preliminary Proceedings 9th European Symposium on Poultry Nutrition*, September 5-9, Jelenia Gora, Poland, pp. 120-131.

Carré, B. & Gomez, J. (1994) Digestibility of water-soluble pectin and organic acid losses in intact or cecectomized adult cockerels. *Poultry Science* 73: 1881-1886.

Carré, B., Florès, M.-P., & Gomez, J. (1995^a) Effects of pelleting, lactose level, polyethylene Glycol 4000, and guar gum compared to pectin on growth performances, energy values, and losses of lactose, lactic acid, and water in chickens. *Poultry Science* 74: 1810-1819.

Carré, B., Gomez, J. & Chagneau, A.M. (1995^b) 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. *British Poultry Science* 36: 611-629.

Carré, B. & Lacassagne, L. (1992) Digestibilities of carbohydrates and matabolizable energy value of peas, faba beans and white lupins in chickens. In: *Proceedings of the 1st European Conference on Grain Legumes*, Association Européenne des Progéagineux, Anger, France. pp. 481-482.

Caspary, W.F. (1992) Physiology and pathophysiology of intestinal absorption. *American Journal of Clinical Nutrition* 55: 299s-308s.

Chateau, N., Deschamps, A.M. & Hadj Sasi, A. (1994) Heterogeneity of bile salts resistance in *Lactobacillus* isolates of a probiotic consortium. *Letter Applied Microbiology* 18: 42-44.

Choct, M. & Annison, G. (1990) Antinutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992^a) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M. & Annison, G. (1992^b) Anti-nutritive effect of wheat pentosans in broiler-chickens: Role of viscosity and gut microflora. *British Poultry Science* 33: 821-834.

Choct, M., Annison, G. & Trimble, R.P. (1992) Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition* 122: 2457-2465.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Classen, H.L. & Bedford, M.R. (1991) The use of enzymes to improve the nutritive value of poultry feeds. In: *Recent Advances in Animal Nutrition.* (Haresign, W. & Cole, D.J.A., eds.) Butterworth-Heineman, Oxford, pp. 95-116.

Classen, H.L., Campbell, G.L., Rosnagel, B.G., Bhatty, R., & Reichert, R.D. (1985) Studies on the use of hulles barley in chick diets: Deleterious effects and methods of alleviation. *Canadian Journal of Animal Science* 65: 725-733.

Classen, H.L., Campbell, G.L. & GrootWassink, W.B. (1988) Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementaiton. *Canadian Journal of Animal Science* 68: 1253-1259.

Coates, M.E. (1976) In: *Digestion in the Fowl.* (Boorman, K.N. & Freeman, C.P., eds.). 1st ed. British Poultry Science Ltd. Edinburgh. pp. 179-191.

Coates, M.E. (1980) The gut micro-flora and growth. In: *Growth in Animals,* (Lawrence T.L.J., eds.), Butterworths, London, pp. 175-188.

Coates, M.E., Cole, C.B., Fuller, R., Houghton, S.B. & Yokota, H. (1981) The gut microflora and the uptake of glucose from the small intestine of the chick. *British Poultry Science* 22: 289-294.

Coates, M.E., Fuller, R. & Harrison, G.F. (1963) A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. *British Journal of Nutrition* 17: 141-150.

Cole, C.B. & Fuller, R. (1984) A note on the effect of host specific fermented milk on the coliform population of the neonatal rat gut. *Journal of Applied Bacteriology* 56: 495-498.

Cooke, R.H. & Bird, F.H. (1973) Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. *Poultry Science* 52: 2276.

Coon, C.N., Leske, K.L., Akavanichan, O. & Cheng, T.K. (1990) Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. *Poultry Science* 69: 787-793.

Corrier, D.E., Hargis, B., Hinton, A. Jr., Lindsey, D., Caldwell, D., Manning, J. & DeLoach, J.R. (1991^a) Effect of anaerobic cecal microflora and dietary lactose on colonization resistance of layer chicks to invasive *Salmonella enteritidis. Avian Diseases* 35: 337-343.

Corrier, D.E., Hinton, A. Jr., Kubena, L.F., Ziprin, R.L. & DeLoach, J.R. (1991^b) Decreased *Salmonella* colonization in turkey poults inoculated with anaerobic cecal microflara and provided dietary lactose. *Poultry Science* 70: 1345-1350.

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

Crabbe, P.A., Nash, D.R., Bazin, H., Eyssen, H. & Heremans, J.F. (1970) Immunohistological observations on lymphoid tissues from conventional and germfree mice. *Laboratory Investigations* 22: 448-457.

Cummings, H.J. (1981) Dietary fibre. *British Medical Bulletin* 37: 65-70.

Cunningham, P. (1989) Dietary lactose treatment may result in lower Salmonella concentration. *Poultry Times*, Dec. 18 p. 3.

Drasar, B.S. (1988) The bacterial flora of the intestine. In: *Role of the gut flora in toxicity and cancer* (Rowland, I.R., ed.), Academic Press, London, pp. 23-38.

Durst, L. (1996) Der Einsatz von Fructo- und Galacto-oligosacchariden in der Broilermast. *Archiv für Geflügelkunde* 60: 160-164.

Eastwood, M.A. (1992) The physiological effect of dietary fiber: an update. *Annual Reviews of Nutrition* 55: 436-442.

Edwards, C.A., Johnson, I.I. & Read, N.W. (1988) Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? *European Journal of Clinical Nutrition* 42: 307-312.

Ellen, R.P., & Gibbons, R.J. (1972) M-protein associated adherence of *Streptococcus pyogenes* to epithelial surfaces: pre-requisite for virulence. *Information and Immunolology* 5: 826-830.

Ewing, W.N. & D.J.A. Cole (1994) The living gut. A Context Publication, Dungannon, N. Ireland.

Eyssen, H. & DeSomer, P. (1963) Effect of antibiotics on growth and nutrients absorption of chicks. *Poultry Science* 42: 1373-1379.

Feighner, S.D. & Dashkevicz, M.P. (1988) Effect of dietary carbohydrates on bacterial cholyltauryl hydrolase in poultry intestinal homogenates. *Applied Environmental Microbiology* 54: 337-342.

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

Fengler, A.I., Pawlik, J.R. & Marquardt, R.R. (1988) Improvement in nutrient retention and changes in excreta viscosities in chicks fed rye-containing diets supplemented with fungal enzymes, sodium taurocholate and panicillin. *Canadian Journal of Animal Science* 68: 483-491.

Ferket, P.R. (1991) Effect of diet on gut microflora of poultry. *Zootechnica:* 44-49.

Fincher, G.B. & Stone, B.A. (1986) Cell walls and their components in ceral grain technology. In: *Advances in Cereal Science and Technology,* Volume 8 (Pomeranz, Y., ed.), Minnesota, AACC, pp. 207-295.

Forbes, M. & Park, J.T. (1959) Growth of germ-free and conventional dietary penicillin and bacterial environment. *Journal of Nutrition* 67: 69.

Freeman, C.P. (1969) Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. *British Journal of Nutrition* 23: 249-262.

Freeman, B.M., Manning, A.C.C., Harrison, G.F. & Coates, M.E. (1975) Dietary aureomycin and the response of the fowl to stressors. *British Poultry Science* 16: 395-401.

Freter, R. & Jones, G.W. (1976) Adhesive properties of *Vibrio cholera*: Nature of the interaction with mucosal surfaces. *Infectuous Immunology* 14: 246.

Fubara, E. & Freter, R. (1973) Protection against bacterial infection by secretory IgA antibodies. *Journal of Immunology* 111: 395-403.

Fuller, R. (1984) Microbial activity in the alimentary tract of birds. *Proceedings of the Nutrient Society* 43: 55-61.

Fuller, R. (1989) Probiotics in man and animals. *Journal Applied Bacteriology* 66: 365-378.

Fuller, R. & Turvey, A. (1971) Bacteria associated with the intestinal wall of the fowl (Gallus domesticus). *Journal of Applied Bacteriology* 34: 617-611.

Fuller, R. & Brooker, B.E. (1974) Lactobacilli which attach to the crop epithelium of the fowl. *American Journal of Clinical Nutrition* 27: 1305-1312.

Fuller, R. & Cole, C.B. (1988) The scientific basis of the probiotic concept. In: *Probiotics - Theory and Applications* (Stark, B.A. & Wilkinson, J.M., eds.) Chalcombe Publications, Marlow, pp. 1-14.

Furuse, M. & Yokota, H. (1985) Effect of the gut microflora on chick growth and utilization of protein and energy at different concentrations of dietary protein. *British Poultry Science* 26: 97-104.

Furuse, M. & Yokota, H. (1984) Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. *British Journal of Nutrition* 51: 255-264.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Gee, J.M., Lee-Finglas, W., Wortley, G.W. & Johnson, J.T. (1996). Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. *Journal of Nutrition* 126: 373-379.

Gitzelmann, R. & Auricchio, S. (1965) The handling of soy alpha-galactosidase by a normal and galactosemic child. *Pedriatrics* 36: 231-232.

Gomez, M.X. & Polin, D. (1974) Influence of cholic acid on the utilization of fats in the growing chicken. *Poultry Science* 53: 773-781.

Gomez, M.X. & Polin, D. (1976) The use of bile salts to improve absorption of tallow in chicks one to three weeks of age. *Poultry Science* 55: 2189-2195.

Goodlad, R.A., Ratcliffe, B.R., Fordham, J.P. & Wright, N.A. (1989) Does dietary fibre stimulate intestinal cell proliferation in germ-free rats? *Gut* 3: 820-825.

Gorbach, S.L. (1990) Lactic acid bacteria and human health. *Annual Medicine* 22: 37-41.

Gordon, H.A. & Bruckner-Kardoss, E. (1961) Effect of normal microbial flora on intestinal surface area. *Americal Journal of Physiology* 201: 175-178.

Green, J. & Kellogg, T.F. (1987) Bile acid concentrations in serum, bile, jejunal contents, and excreta of male broiler chicks during the first six weeks posthatch. *Poultry Science* 66: 535-540.

Grill, J.P., Manginot-Dürr, C., Schneider, F. & Ballongue, J. (1995) Bifidobacteria and probiotic effects: action of *bifidobacterium* species on conjugated bile salts. *Current Microbiology* 31: 23-27.

GrootWassink, J.W.D., Campbell, G.L. & Classen, H.L. (1989) Fractionation of crude pentosanase (arabinoxylanase) for improvement of the nutritional value of rye diets for broiler chickens. *Journal of Food Agriculture* 46: 289-300.

Hampson, D.J. (1986) Alterations in piglet small intestinal structure at weaning. *Research in Veterinary Science* 40: 3.

Havenaar, R. & Spanhaak, S. (1994) Probiotics from an immunological point of view. *Current Opinion in Biotechnologie* 5: 320-325.

Havenstein, G.B., Ferkel, P.R., Scheideler, S.E. & Larson, B.T. (1994^a) Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* 73: 1785-1794.

Havenstein, G.B., Ferkel, P.R., Scheideler, S.E. & Rives, D.V. (1994^b) Carcass composition, and yield of 1991 vs 1957 broilers when fed "typical" 1957 and 1991 broiler diets. *Poultry Science* 73: 1795-1804.

Hedge, S.N., Rolls, B.A. & Coates, M.E. (1982) The effects of the gut microflora and dietary fibre on energy utilization by the chick. *British Journal of Nutrition* 48: 73-80.

Heneghan, J.B. (1965) Imbalance of the normal microbial flora: The germfree alimentary tract. *American Journal of Digestive Diseases* 10: 864-869.

Heneghan, J.B. (1979) Enterocyte kinetics, mucosal surface area and mucis in gnotobiotes. In: *Clinical and Experimental Gnotobiotes* (Fludnes, T., Heil, H., Niethammer, D. & Pfjeger, H., eds.) Gustov Fischer Verlag, Stuttgart, pp. 19-29.

Hentges, D.J. (1980) Gut flora and disease resistance. In: *Probiotics - The Scientific Basis* (Fuller, R., ed.) Chapman & Hall, London, pp. 87-110.

Hesselman, K. & Aman, P. (1986) The effect of ß-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. *Animal Feed Science and Technology* 15: 83-93.

Hesselman, K., Elwinger, K., Nilsson, M. & Thomke, S. (1981) The effects of ß-glucanase supplementation, stage of ripeness and storage treatment of barley in diets fed to broiler chickens. *Poultry Science* 60: 2664-2671.

Hidaka, H., Edida, T., Takazawa, T., Tokunga, T. & Tashiro, Y. (1986) Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* 5: 37-50.

Hinton, A., Jr., Corrier, D.E., Norman, J.O., Beier, R.C. & DeLoach, J.R. (1990) Biological control of *Salmonella typhimurium* in young chickens. *Avian Diseases* 34: 626-633.

Hinton, A., Jr., Corrier, D.E., Ziprin, R.L., Spates, G.E. & DeLoach, J.R. (1991) Comparison of the efficacy of cultures of cecal anaerobes as inocula with or without dietary lactose. *Poultry Science* 70: 67-73.

Hirsch, D.C. (1980) Microflora, mucosa and immunity: Disruption of the normal microflora. In: *Veterinary Gastroenterology* (Anderson, N.V., ed.) Lea and Febiger, Philadelphia, PA, pp. 208-224.

Hofmann, A.F. & Mysels, K.J. (1992) Bile acid solubility and precipitation *in vitro* and *in vivo*: the role of conjugation, pH and Ca^{2+} ions. *Journal of Lipid Research* 33: 617-626.

Hofshagen, M. & Kaldhusdal, M. (1992) Barley inclusion and avoparcin supplementation in broiler diets. 1. Effect on small intestinal bacterial flora and performance. *Poultry Science* 71: 959-969.

Hulan, H.W. & Bird, F.H. (1972) Effect of fat level in isonitrogenous diets on the composition of avian pancreatic juice. *Journal of Nutrition* 102: 459-468.

Hylemond, P.B. (1985) Metabolism of bile acids in intestinal microflora In: *Sterols and bile acids: New Comprehensive Biochemistry* (Danielsen, H. & Sjövall, J., eds.) Elsevier Science Publishers B.V., pp. 331-343.

Ikegami, S., Tsuchihashi, F., Harada, H., Tsuchihashi, N., Nishide, E. & Innami, S. (1990) Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. *Journal of Nutrition* 120: 353-360.

Iñarrea, P., Simon, M., Manzano, M. & Palacios, J. (1989) Changes in the concentration and composition of biliary and serum bile acids in the young domestic fowl. *British Poultry Science* 30: 353-359.

Jacobs, L.R. (1983) Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: a comparison of oat bran, pectin, and guar with total fiber deprivation. *American Journal of Clinical Nutrition* 37: 954-960.

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

Johnson, I.T., Gee, J.M. & Mahoney, R.R. (1984) Effect of dietary supplements of guar gum or cellulose on intestinal cell proliferation enzyme levels and sugar transport in the rat. *British Journal of Nutrition* 52: 477-487.

Johnson, I.T. & Gee, J.M. (1986) Gastrointestinal adaption in response to soluble non-available polysaccharides in the rat. *British Journal of Nutrition* 55: 497-505.

Just, A., Fernande, J.A. & Jorgensen, H. (1983) The net energy value of diets for growth in pigs in relation to the fermentative processes in the digestive tract and the site of absorption of the nutrients. *Livestock Production Science* 10: 171-186.

Juste, C., Corring, T. & Breant, Ph. (1979) Excrétion billiaire chez le porc: niveau et response au repas. *Annual Biology for Animal Biochemy and Biophysiology* 19: 79-90.

Juste, C., Demarne, Y. & Corring, T. (1983) Response of bile flow, biliary lipids and bile acid pool in the pig to quantitative variations in dietary fat. *Journal of Nutrition* 113: 1691-1701.

Kaldhusdhal, M. & Hofshagen, M. (1992) Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. *Poultry Science* 71: 1145-1153.

Ketels, E. & De Groote, G. (1988) The nutritional value for broilers of fats characterized by short-chain fatty acids as affected by level of inclusion and age. *Animal Feed Science and Technology* 22: 105-118.

Koninkx, J.F.J.G., Stemerdink, A.F.E., Mirck, M.H., Egberts, H.J.A., Van Dijk, J.E. & Mouwen, J.M.V.M. (1988) Histochemical changes in the composition of mucins in goblet cells during methotrexate-induced mucosal atrophy in rats. *Experimental Pathology* 34: 125-133.

Koopman, J.P. (1984) Kolonisatie resistentie van het maagdarmkanaal. *Netherlands Journal of Veterinary Science* 109: 1017-1026.

Krogdahl, A. (1985) Digestion and absorption of lipids in poultry. *Journal of Nutrition* 115: 675-685.

Kussaibati, R., Guillaume, B. & Leclerq, B. (1982^a) The effect of endogenous energy, type of diet and addition of bile salts on true metabolizable energy values in young chick. *Poultry Science* 61: 2218-2223.

Kussaibati, R., Guillaume, B., Leclerq, B. & Lafont, J.P. (1982^b) Effects of intestinal microflora and added bile salts on metabolizable energy and digestibility of satureated fats in the chicken. *Archiv für Geflügelkunde* 46: 42-46.

Larsen, F.M., Moughan, P.J. & Wilson, M.N. (1993) Dietary fiber viscosity and endogenous protein excretion at the terminal ileum of growing rats. *Journal of Nutrition* 123: 1898-1904.

Lee, A. (1985) Neglected niches: The microbial ecology of the gastrointestinal tract. *Advanced Microbial Ecology* 8: 115-162.

Leske, K.L., Curtis, J.J. & Coon, C.N. (1993) Effect of oligosaccharides additions on nitrogen-corrected true metabolizable energy of soy protein concentrate. *Poultry Science* 72: 664-668.

Leske, K.L., Zhang, B. & Coon, C.N. (1995) The use of low alpha-galactoside protein products as a protein source in chicken diets. *Animal Feed Science and Technology* 54: 275-286.

Lim, S. & Low, F.N. (1977) Scanning electron microscopy of the developing canal of the chick. *American Journal of Anatomy* 150: 149-154.

Lloyd, A.B., Cummings, R.B. & Kent, R.D. (1977) Prevention of *Salmonella typhimurium* infection in poultry by pretreatment of chicks and poults with intestinal extracts. *Australian Veterinarian Journal* 53: 82-87.

Longstaff, M.A., Knox, A. & McNab, J.M. (1988) Digestibility of pentose sugars and uronic acids and their effect on chick weight gain and ceacal size. *British Poultry Science* 29: 379-393.

Lukey, T.D. (1987) Microbic effects of low digestibility carbohydrates. In: *Low Digestible Carbohydrates* (D.C. Leewwater, Feron, V.J. & Hermus, R.J.J., eds.) Wageningen: Pudoc Press, the Netherlands, pp. 23-33.

MacKinnon, J.D. (1985) The efficacy, benefits and general use of antimicrobial growth promoters in animal nutrition. In: *Proceedings of the Symposium "Criteria and Methods for the Microbiological Evaluation of Growth Promters in Animal Feeds"* (Helmuth, R. & Bulling, E., eds.) Berlin, pp. 5-27.

March, B.E., Soong, R. & MacMillen, C. (1978) Growth rate, feed conversion, and dietary metabolizable energy in response to virginiamycin supplementation of different diets. *Poultry Science* 57: 1346-1350.

Marquardt, R.R., Ward, A.T. & Misir, R. (1979) The retention of nutrients by chicks fed rye diets supplemented with amino acids and penicillin. *Poultry Science* 58: 631-640.

Masuda, N. (1981) Deconjugation of bile salts by *Bacteroides* and *Clostridium*. *Microbiology and Immunology* 25: 1-11.

May, M.B. (1990) Industrial pectin: sources, production and applications. *Carbohydrate Polymeres* 12: 79-99.

Mead, G.C. (1989) Microbes of the Avian cecum: types present and substrates utilized. *The Journal of Experimental Zoology Supplement* 3: 48-54.

Mead, G.C. & Adams, B.W. (1975) Some observations on the caecal micro-flora of the chick during the first two weeks of life. *British Poultry Science* 16: 169-176.

Miles, R.D. (1993) Manipulation of micro-flora in the digestive tract: natural ways to prevent colonisation by pathogens. In: *Biotechnology in the Feed Industry* (Lysons, T.P., ed.) Alltech Technical Publications. Nicholasville, Kentucky, pp. 133-150.

Mitsuoka, T., Hidaka, H. & Eida, T. (1987) Effect of fructooligosaccharides on intestinal microflora. *Nahrung* 31: 427-436.

Mollah, Y. & Annison, E.F. (1981) Wheat: Maize interactions in the bioassay of metabolizable energy in poultry diets in relation to the digestibility of starch. *Proceedings of the Nutrition Society of Australia* 6: 138-143.

Moran, E.T. Jr. (1982) The gastrointestinal system. Comparative nutrition of fowl and swine. University of Geulph.

Morishita, Y., Fuller, R. & Coates, M.W. (1982) Influence of dietary lactose on the gut flora of chicks. *British Poultry Science* 23: 349-359.

Morland, B. & Midtvedt, T. (1984) Phagocytosis, peritoneal influx and enzyme activities in peritoneal macrophages from germfree, conventional and ex-germfree mice. *Infectious Immunology* 44: 750-752.

Muramatsu, T., Kodama, H., Morishita, T., Furuse, M. & Okumura, J. (1991) Effect of intestinal microflora on digestible energy and fiber digestion in chickens fed a high-fiber diet. *American Journal of Veterinary Research* 52: 1178-1181.

Nair, P.P., Gordan, M. & Reback, J. (1967) The enzymatic cleavage of the carbon-nitrogen bond in 3α, 7α, 12α-trihydroxy-5ß-cholan-24-oylglycine. *Journal of Biology and Chemistry* 242: 7-11.

Nelson, F.E., Jensen, L.S. & McGinnis, J. (1963) Studies on the stimulation of growth by dietary antibiotics. 2. Effect of antibiotics on metabolizable enery of the diet. *Poultry Science* 42: 909-912.

Nir, J., Nitsan, Z. & Mahagna, M. (1993) Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. *British Poultry Science* 34: 523-532.

Nitsan, Z., Ben-Aviaham, G., Zoref, Z. & Nir, J. (1991) Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *British Poultry Science* 32: 515-523.

Norstrandt, A.C. (1990) Bacteria and septicemia in small animal patients. *Problems in Veterinary Medicine* 2: 348-361.

Nurmi, E. & Rantala, M. (1973) New aspects of salmonella infection in broiler produktion. *Nature* 241: 210-211.

Ochi, Y., Mitsuoka, T. & Sega, T. (1964) Untersuchungen über die Darmflora des Huhnes II Mitteilung: Die Entwicklung der Darmflora von Küken bis zum Huhn. *Zentralblad der Bakteriologie und Parsitenkunde Infectionskriege und Hygiene (Abt. I)* 193: 80-95.

Ofek, I., Mirelman, D. & Sharon, N. (1977) Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* 256: 623-625.

Oyofo, B.A., Deloach, J.R., Corrier, D.E., Norman, J.O., Ziprin, R.L. & Mollenhauer, H.H. (1989^a) Prevention of *Salmonella typhimurium* colonisation of broilers with D-mannose. *Poultry Science* 68: 1357-1360.

Oyofo, B.A., DeLoach, J.R., Corrier, D.E., Norman, J.O., Ziprin, R.I. & Mollenhauer, H.H. (1989^b) Effects of carbohydrates on Salmonella typhimurium colonization in broiler chickens. *Avian Diseases* 33: 531-534.

Oyofo, B.A., Droleskey, R.E., Norman, J.O., Mollenhauer, H.H., Ziprin, R.L., Corrier, D.E. & DeLoach, J.R. (1989^c) Inhibition by mannose of *in vitro* colonization of chicken small intestine by Salmonella typhimurium. *Poultry Science* 68: 1351-1356.

Pappo, I., Bercovier, H., Berry, E.M., Haviv, Y., Gallily, R. & Freund, H.R. (1992) Polymyxin B reduces total parenteral nutrition-associated hepatic steatosis by its antibacterial activity and by blocking deleterious effects of lipopolysaccharide. *Journal of Parenteral and Enteral Nutrition* 16: 529-532.

Parsons, H.G. (1986) Clinical aspects of fat malabsorption. In: *Fat absorption vol 1* (Kuksis, A., ed.), CRC Press, Boca Raton, Florida, pp. 261-293.

Patel, M.B., McGinnis, J. & Pubois, M.H. (1981) Effect of dietary cereal grain, citrus pectin and guar gum on liver fat in laying hens and young chicks. *Poultry Science* 60: 631-636.

Patel, M.B., Jami, M.S. & McGinnis, J. (1980) Effect of gamma irradiation, penicillin and or pectic enzyme on chick growth depression and fecal stickiness by rye, citrus pectin and guar gum. *Poultry Science* 59: 2105-2110.

Perdigon, G., Nader de Macias, M.E., Alvarez, S., Medici, M., Oliver, G. & Pesce de Ruiz Holgado, A. (1986) Effect of a mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* administered orally in the immune system in mice. *Journal of Food Protection* 49: 986-989.

Perdigon, G., Nader de Macias, M.E., Alvarez, S., Oliver, G. & Pesce de Ruiz Holgado, A. (1987) Enhancement of immune response in mice fed with Streptococcus thermophilus and Lactobacillus acidophilus. *Journal of Dairy Science* 70: 919-929.

Perdigon, G., Alvarez, S., Nader de Macias, M.E., Roux, M.E. & Pesce de Ruiz Holgado, A. (1990) The oral administration of lactic acid bacteria increase the mucosal intestinal immunity in response to enteropathogens. *Journal of Food Protection* 53: 404-410.

Poksay, K.S. & Schneeman, B.O. (1983) Pancreatic and intestinal response to dietary guar gum in rats. *Journal of Nutrition* 113: 1544-1549.

Polin, P., Wing, T.C., Ki, P. & Pell, K.E. (1980) The effect of bile acids and lipase and absorption of tallow in young chicks. *Poultry Science* 59: 2738-2743.

Pollman, D.S. (1986) Probiotics in pig diets. In: *Recent Advances in Animal Nutrition* (Haresign, W. & Cole, D.J.A., eds.) Butterworths, London.

Pollman, D.S., Danielson, D.M., Wren, W.B., Peo, E.R. Ir. & Shahani, K.M. (1980) Influence of

Lactobacillus acidophilus inoculum on gnotibiotic and conventional pigs. *Journal of Animal Science* 51: 629-637.

Rantala, M. (1974) Cultivation of a bacterial flora able to prevent colonization of *salmonella infantis* in the intestine of broiler chickens and its use. *Acta Pathologica et Microbiologica Scandinavia* B82: 75-80.

Ratcliffe, B. (1985) The influence of the gut microflora on the digestive processes. In: *Digestive Physiology in the Pig* (Just, A., Jorgensen, H. & Fernandez, J.A., eds.) Copenhagen, National Institute of Animal Science, pp. 245-267.

Robertson, J.A. (1988) Physiochemical characteristics of food and the digestion of starch and dietary fibre during gut transit. *Proceedings of the Nutrition Society* 43: 143-152.

Robertson, J.A. & Eastwood, M.A. (1981) An examination of factors which may affect the water holding capacity of dietary fibre. *British Journal of Nutrition* 45: 83-88.

Rogel, A.M., Annison, E.F., Bryden, W.L. & Balnave, D. (1987) The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research* 38: 639-649.

Rolfe, R.D. (1991) Population dynamics of the intestinal tract. In: *Colonization control of human enteropathogens in poultry* (Blankenship, L.C., ed.) Academic Press; Inc. San Diego, CA, pp. 59-75.

Rolls, B.A., Turvey, A. & Coates, M.E. (1978) The influence of the gut microflora and dietary fibre on epithelial cell migration in the chick intestine. *British Journal of Nutrition* 39: 91-98.

Rosebury, T. (1962) Micro-organisms indigenous to Man. McGraw-Hill Book Co. Inc. New York, p. 435.

Rowland, I.R., Mallett, A.K. & Wise, A. (1985) The effect of diet on the mammalian gut flora and its metabolic activities. *CRC Critical Review and Toxicology* 16: 31-103.

Rowland, I.R. & Walker, R. (1983) The gastro-intestinal tract in food toxicology. In: *Toxic hazards in foods* (Conning, D.M. & Landsdown, A., eds.), Croom-helm, London, pp. 183-274.

Sakata, T. (1987) Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal throphic factors. *British Journal of Nutrition* 58: 95-103.

Salanitro, J.P., Blake, I.G. & Muirhead, P.A. (1974) Studies on the cecal microflora of commercial broiler chickens. *Applied Microbiology* 28: 439-447.

Salanitro, J.P., Blake, I.G., Muirhead, P.A., Maglio, M. & Goodman, J.R. (1978) Bacteria isolated from the duodenum, ileum and cecum of young chicks. *Applied and Environmental Mcirobiology* 35: 782-790.

Salih, M.E. Classen, H.L. & Campbell, G.L. (1991) Response of chickens fed on hull-less barley to dietary ß-glucanase at different ages. *Animal Feed Science and Technology* 33: 139-149.

Salter, D.N. & Coates, M.E. (1974) The utilization of protein and excretion of uric acid in germ-free and conventional chicks. *British Journal of Nutrition* 31: 307-318.

Satchithanandam, S., Vargofcak-Apker, M., Calvert, R.S., Leeds, A.R. & Cassidy, M.M. (1990) Alteration of gastro-intestinal mucin by fibre feeding in rats. *Journal of Nutrition* 120: 1179-1184.

Savage, D.C. (1972) Associations and physiological interactions of indigenous micro-organisms and gastro-intestinal epithelia. *American Journal of Clinical Nutrition* 25: 1372-1379.

Savage, D.C. (1977) Microbial ecology of the gastro-intestinal tract. *Annual Reviews Microbiology* 31: 107-133.

Savage, D.C. (1983) Mechanisms by which indigenous microorganisms colonize gastro-intestinal epithelial surfaces. *Progression in Food and Nutrition Science* 7: 65-74.

Savage, D.C. (1985) Effects of host animals of bacteria adhering to epithelial surfaces. In: *Bacterial Adhesion* (Savage, D.C. & Fletcher, M., eds.). Plenum, New York, pp. 437-463.

Savage, D.C. (1986) Gastro-intestinal micro-flora in mammalian nutrition. *Annual Review Nutrition* 6: 155-178.

Schneeman, B.D. 91982) Pancreatic and digestive function. In: *Dietary Fibre in Health and Disease* (Vahoung, G.V. & Kritchevsky, D. eds.) Plenum Press, New York, pp. 73-83.

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

Schutte, J.B. (1991) Nutritional value and physiological effects of D-xylose and L-arabinose in poultry and pigs. PhD thesis, Agricultural University Wageningen, the Netherlands.

Schutte, J.B., De Jong, J., Polziehn, R. & Verstegen, M.W.A. (1991) Nutritional implications of D-xylose in pigs. *British Journal of Nutrition* 66: 83-93.

Schutte, J.B., De Jong, J., Van Weerden, E.J. & Van Baak, M.J. (1992^a) Nutritional value of D-xylose and L-arabinose for broiler chickens. *British Poultry Science* 33: 89-100.

Schutte, J.B., De Jong, J., Van Weerden, E.J. & Tamminga, S. (1992^b) Nutritional implications of L-arabinose in pigs. *British Journal of Nutrition* 68: 195-207.

Schutte, J.B., Geerse, C. & De Jong, J. (1993^a) Effect of ß-glucanase supplementation to a barley-based diet on broiler chick performance. *Proceedings World Poultry Science Association*, Jelenia Gora, Poland, pp. 490-492.

Schutte, J.B., Geerse, C. & De Jong, J. (1993^b) Effect of enzyme supplementation to wheat-based diets on broiler chicken performance. *Proceedings Enzyme Congress*, Switzerland, pp. 133-136.

Setchell, K.D.R., Lawson, A.M., Blackstock, E.J. & Murphy, G.M. (1982) Diurnal changes in serum unconjugated bile acids in normal man. *Gut* 23: 637-642.

Sharon, N. (1987) Bacterial lectins, cell-cell recognition and infectious disease. *FEBS Letters* 217: 145-157.

Siddons, R.C. & Coates, M.E. (1972) The influence of the intestinal microflora on dissaccharidase activities in the chick. *British Journal of Nutrition* 27: 101-112.

Smith, H.W. (1965) The development of the flora of the alimentary tract in young animals. *Journal of Pathology and Bacteriology* 90: 495-513.

Smithson, K.W., Millar, D.B., Jacobs, L.R. & Gray, G.M. (1981) Intestinal diffusion barrier: unstirred water layer or membrane surface mucous coat. *Science* 214: 1241-1244.

Smits, C.H.M. (1996) Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. PhD thesis, Agricultural University Wageningen, the Netherlands.

Smits, C.H.M. & Annison, G. (1996) Non-starch plant polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. *World's Poultry Science Journal* 52: 203-221.

Stremmel, W. (1987) Absorption of fat and fat-soluble vitamins. In: *Structure and function of the small intestine* (Caspary, W.F., ed.) Amsterdam: Excerte Medica pp. 175-184.

Tellez, G., Dean, C.E., Covrier, D.E., DeLoach, J.R., Jaeger, L. & Hargis, B.M. (1993) Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella enteritidis* organ invasion in leghorn chicks. *Poultry Science* 72: 636-642.

Treviño, J., Centeno, C., Brenes, A., Yuste, P. & Rubio, L. (1990) Effect of dietary oligosaccharides on the digestion of pea starch by growing chicks. *Animal Feed Science and Technology* 30: 313-319.

Trowell, H., Southgate, D.A.T., Wolever, T.M.S., Leeds, A.R., Gassull, M.A. & Jenkins, D.J.A. (1976) Dietary fibre redefined. *Lancet* 1: 967.

Truscott, R.B. & Al-Sheikhly, F. (1977) Reproduction and treatment of necrotic enteritis in broilers. *American Journal of Veterinary Research* 38: 857-861.

Van Es, A.J.H. (1974) Energy utilization of low digestibility carbohydrates. In: *Low digestibility carbohydrates* (Leegwater, D.C., Feron, V.J. & Hermus, R.J.J., eds.). Wageningen: Pudoc Press, pp. 121-127

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

Van der Waaij, D., Berghuis de Vries, J.M. & Lekkerkerk van der Wees, J.E.C. (1971) Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *Journal of Hygiene* 69: 405-411.

Veldman, A. & Vahl, H.A. (1994) Xylanase in broiler diets with differences in characteristics and content of wheat. *British Poultry Science* 35: 537-550.

Visek, W.J. (1978). The mode of growth promotion by antibiotics. *Journal of Animal Science* 46: 1447-1469.

Viveros, A., Brenes, A., Pizarro, M. & Castano, M. (1994) Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Animal Feed Science and Technology* 48: 237-251.

Vohra, P. & Kratzer, F.H. (1964) Growth inhibitory effect of certain polysaccharides for chickens. *Poultry Science* 43: 1164-1170.

Vukic Vranjes, M. & Wenk, C. (1995) Influence of dietary enzyme complex on the performance of broilers fed on diets with and without antibiotic supplementation. *British Poultry Science* 36: 265-275.

Wagner, D.D. & Thomas, O.P. (1977) A rye type growth depression of chicks fed pectin. *Poultry Science* 56: 615-619.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

Waldroup, A.L., Skinner, J.T., Hierholzer, R.E. & Waldroup, P.W. (1993) An evaluation of fructooligosaccharide in diets for broiler chickens and effects on salmonellae contamination of carcasses. *Poultry Science* 72: 643-650.

Wang, L., Newman, R.K., Newman, C.W. & Hofer, P.J. (1992) Barley ß-glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. *Journal of Nutrition* 122: 2292-2297.

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

Ward, A.T. & Marquardt, R.R. (1987) Antinutritional activity of a water-soluble pentosan-rich fraction from rye grain. *Poultry Science* 66: 1665-1674.

Westergaard, H. & Dietschy, J.M. (1976) The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell *Journal of Clinical Investigations* 58: 97-108.

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1981) The viscosity interaction of barley ß-glucan with Trichoderma viride cellulase in the chick intestine. *Poultry Science* 62: 853-862.

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1983) Viscosity of ß-D-glucan as a factor in the enzymatic improvement of barley for chicks. *Poultry Science* 62: 853-862.

Wiggins, H.S. (1984) Nutritional value of sugars and related compounds undigested in the small gut. *Proceedings of the Nutrition Society* 43: 69-75.

Wilson, F.A. & Dietschy, J.M. (1974) The intestinal unstrirred layer: its surface area and effect on active transport kinetics. *Biochimica Biophysica Acta* 366S: 112-126.

Wyatt, G.M., Horn, W., Gee, J.M. & Johnson, I.T. (1988) Intestinal microflora and gastrointestinal adaptation in the rat in response to non-digestible dietary polysaccharides. *British Journal of Nutrition* 60: 197-207.

Chapter 2

Effects on chick performance and nutrient digestibility of an endo-xylanase supplementation to a wheat and rye based diet in relation to fat source

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Abstract

A study with growing chicks investigated the effects of an inclusion of an endo-xylanase preparation (LYXASAN®) to a wheat- and rye-based diet on performance and nutrient digestibility in relation to the fat source. The basal diet contained 500 g wheat and 100 g rye/kg of diet. The basal diet was supplemented with 65 g soya oil/kg or 60 g blended animal fat and 5 g soya oil/kg. Endo-xylanase added to the soya oil diet did not affect weight gain, but there was a numerical improvement in food conversion efficiency which was not statistically significant. When the endo-xylanase preparation was added to the blended animal fat diet, both weight gain and food utilisation were improved by 9.5 and 6.0%, respectively ($P < 0.05$). Digestibilities of organic matter, crude fat, crude fibre and NFE were not significantly affected by adding endo-xylanase to the soya oil diet. However, when endo-xylanase was included in the blended animal fat diet, digestibility of organic matter, crude fat, crude fibre and NFE were improved ($P < 0.05$). The improvement in fat digestibility was the most pronounced amounting to 9.4%. Nitrogen retention and metabolisable energy content were improved significantly by the addition of an endo-xylanase to the animal fat diet ($P < 0.05$), by 6.6% and 6.5% respectively. From the results of this study, it can be concluded that the effects on chick performance and nutrient digestibility of a dietary endo-xylanase in a wheat- and rye-based diet are influenced to a considerable degree by the type of fat in the diet.

Introduction

It is well established that the non-starch polysaccharides (NSP) of rye and wheat have anti-nutritive properties in broiler chicks. The anti-nutritive effects of rye (Antoniou *et al.*, 1981; Ward and Marquardt, 1983) and wheat (Choct and Annison, 1990; 1992) are related to the presence of viscous arabinoxylans (pentosans) in both cereals. Rye contains greater proportions of pentosans (100 g/kg dry weight, Antoniou *et al.*, 1981) than wheat (50 - 80 g/kg dry weight, Annison, 1990). The anti-nutritive effect of rye is also more pronounced than that of wheat (Petterson and Åman, 1988; 1989). Several investigators have shown that the addition of enzymes (xylanases) to rye-based (GrootWassink *et al.*, 1989; Bedford *et al.*, 1991; Bedford and Classen, 1993) and wheat-based diets (Annison, 1992; Schutte *et al.*, 1993) can eliminate the anti-nutritive effect of the pentosans in chicks.

An increase in digesta viscosity is accepted as the primary mechanism by which pentosans reduce the digestibility of nutrients and, therefore, depress performance. Fat absorption in particular is negatively affected (Antoniou *et al.*, 1981; Campbell *et al.*, 1983; Choct and Annison, 1992). Therefore, energy utilisation is affected by the anti-nutritive effect of pentosans in broiler chick diets containing saturated fats to a greater degree than that of protein (Fengler and Marquardt, 1988; Choct and Annison, 1992). Antoniou and Marquardt (1982) demonstrated an interaction between fat source and the anti-nutritive effects of rye on broiler performance. These investigators showed a greater decrease in fat digestibility in rye fed chicks when tallow was included in the diet than when the fat source was soya oil. Ward and Marquardt (1983) related this effect to the degree of saturation and chain length of fats. The response of chicks fed a wheat- and/or rye-based diet containing supplemental enzyme might therefore be affected by the type of added dietary fat. The purpose of the present study was to investigate the effects on chick performance and nutrient digestibility of endo-xylanase addition to a wheat- and rye-based diet containing either soya oil or blended animal fat.

Materials and methods

Experiment 1: Performance

This experiment was designed to compare the effects of endo-xylanase supplementation and type of dietary fat on growth, food intake and food conversion efficiency of broiler chicks. A basal diet containing 500 g wheat/kg and 100 g rye/kg was used. The basal diet was either supplemented with 65 g soya oil/kg or 5 g soya oil and 60 g blended animal fat/kg, respectively. A commercial soya oil low in lecithin was used in the experiment. The blended animal fat was a mixture of poultry-fat, lard and beef tallow. Before preparing the diet, the fatty acid composition of both fat sources was determined (Table 1). The difference in calculated ME value between the two diets was corrected with tapioca and wood cellulose. The composition of the two diets is presented in Table 2 and calculated to be adequate in all nutrients according to the National Research Council (1994). Additives such as antibiotics and coccidiostats which may interfere with dietary xylanases, were omitted from the diets. The two diets were tested without or with an endo-xylanase enzyme supplementation, at a dose level of 100 mg/kg. The endo-xylanase (LYXASAN®, 40%) was supplied by Gist- brocades and had an activity of 28,000 EXU/g.

Table 1. *Fatty acid composition of the fat sources (soya oil and blended animal fat) used (percentage)*

Fatty acids	Soya oil	Animal fat
C10:0 capric acid	-	0.3
C12:0 lauric acid	-	1.3
C14:0 mystiric acid	0.1	2.0
C14:1	-	0.1
C15:0	-	0.4
C15:1	-	0.1
C16:0 palmitic acid	10.9	22.6
C16:1	0.1	2.6
C17:0	0.1	1.0
C17:1	0.1	0.4
C18:0 stearic acid	3.8	14.6
C18:1 oleic acid	21.9	38.8
C18:2 linolic acid	54.4	11.1
C18:2 miscellaneous	0.7	0.4
C18:3 linoleic acid	6.0	0.9
C18:3 miscellaneous	0.5	-
C20:0 arachic acid	0.3	0.4
C20:1	0.7	0.7
C20:2	-	0.1
C20:4 arachidonic acid	-	0.1
C22:0	0.3	0.5
miscellaneous	0.1	1.6

One-day-old male Ross broiler chicks were used. The birds were housed in 3-tiered, electrically heated battery cages with a floor space of 975 cm^2 and wire floors. The cages were situated in an insulated room with controlled temperature and humidity. Chicks were subjected to continuous fluorescent illumination. Each treatment group was allocated at random allocated to 6 cages with 15 birds in each. The experimental diets were fed for a period of 21 d (1 to 21 d of age). At the end of the trial, chicks were weighed individually. Food consumption and efficiency of food utilization were determined for each cage. Diets were fed (as pellets; pellet temperature 60 °C) *ad* *libitum*. Water was also available *ad libitum* from an automatic device.

Table 2. *Composition of the experimental diets (g/kg)*

Ingredient	Soya oil	Animal fat
Wheat (120 g/kg CP)	500	500
Rye (90 g/kg CP)	100	100
Soya oil	65	5
Animal fat	0	60
Tapioca	3	25
Cellulose	27.3	5.3
Soyabean meal (470 g/kg CP)	202	202
Herring meal (730 g/kg CP)	15	15
Meat meal tankage (580 g/kg CP)	40	40
Feather meal, hydr. (820 g/kg CP)	15	15
Vitamin/mineral premix*	10	10
Ground limestone	8.7	8.7
Mono-calcium phosphate	7.1	7.1
Salt	3	3
L-lysine HCl	1.9	1.9
DL-methionine	2	2
Calculated nutrient content		
ME, MJ/kg	12.85	12.97
Crude protein, g/kg	216	216
Crude fat, g/kg	86	86
Calcium, g/kg	8.6	8.6
Phosphorus, g/kg	6.6	6.6
Lysine, g/kg	12.1	12.1
Methionine, g/kg	9.0	9.0

* Supplied per kilogram of diet: riboflavin, 4 mg; niacin amid, 40 mg; d-pantothenic acid, 12 mg; choline chloride, 500 mg; cyano cobalamin, 15 µg; Dl-α-tocopheryl acetate, 20 mg; menadione, 5 mg; retinyl acetate, 3.44 mg; cholecalciferol, 50 mg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.7H_2O$, 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 100 mg; $ZnSO_4$.H2O, 150 mg; $Na_2SeO_3.5H_2O$, 0.15 mg; KJ, 5 mg; $CoSO_4.7H_2O$, 1 mg; and anti-oxidant (ethoxyquin), 100 mg.

Experiment 2: Digestibility and energy value

This experiment was conducted to determine the digestibility of nutrients and the metabolisable energy of the diets involved in the growth trial. In addition, the dry matter contents of the droppings were determined. The digestibility trial was performed with birds from experiment 1. During the digestibility trial, 15 g Diamol/kg, an insoluble ash (Franz Bertram GmbH, Hamburg), was included in the experimental diets as an indicator. The same diets as used in experiment 1 were fed to the birds. Thirty-two birds (21 d of age) from each of the four preceding treatment groups were selected in such a way that the differences in body weight between these groups at the end of the growth trial were maintained. Each treatment group included 4 cages, each with 8 birds.

The birds were housed under conditions similar to those described in the growth trial. The digestibility study included a pre-test period of 5 d (age period 21 to 26 d) and a test period of 4 d (26 to 29 d). During the test period, droppings were collected quantitatively from plastic trays every day. Contaminations, such as down and scales, were carefully removed and the droppings stored in closed containers at -20 °C. During the day (08.00-20.00 h), droppings were collected over 4 h periods, and during the night (20.00-08.00 h) over one 12 h period. Day and night droppings were stored separately.

Birds were deprived of food 6 h before the start of the test period and 6 h before the end of the test period. During the digestibility study, diets were available *ad libitum* and food intake during the test period was determined daily for each cage of 8 birds. Consumption of water, which was also available *ad libitum* from a trough, was measured for each cage over a 12 h period. Water intake was measured as the difference in the volume of water in the pans at the beginning and the end of the treatment period. Digestibility of nutrients was determined only in droppings collected during day time. This procedure was based on the results of previous studies (Van der Klis, 1995, personal communication) indicating that there were no significant differences in digestibility when droppings were collected over a 12 h or over a 24 h period each day. Differences in food intake and droppings production between day and night were corrected on the basis of the recovery of Diamol.

The 4 experimental diets and the excreta were analysed for dry matter, nitrogen, fat and gross energy (GE). The AME of each diet was calculated from the difference between GE intake and GE losses in the droppings. The AME values were corrected to zero nitrogen balance (AME_n). The correction factor of 34.39 kJ/g of retained nitrogen, proposed by Hill and Anderson (1958), was used.

Chemical analysis

An IKA-C4000 adiabatic calorimeter was used to determine the gross energy content of the diets and droppings. The nitrogen content of the diets and droppings were determined by the Kjeldahl procedure (AOAC, 1984) using an automatic analyzer

(Technicon Instruments Systems, Tarrytown, NY). Crude fat was determined after treatment for 1 h with 4 M hydrochloride acid and drying for 3 h under vacuum at 100°C, followed by 8 h extraction with hexane. Amino acids, with the exception of methionine, cystine and tryptophan, were determined on an automatic analyser, after acid hydrolysis (6M hydrochloride acid for 22 h at 100°C) according to Slump (1969), of the diets and the individual droppings samples. Samples that were analysed for methionine and cystine were subjected to performic acid oxidation before hydrolysis. Tryptophan was not determined. For dry matter determinations the samples were dried in an oven at 101 °C. Ash was determined by incineration at 550°C for 4 h. nitrogen-free extract (NFE) was calculated as: dry matter - (ash + crude protein + crude fat + crude fibre).

Statistical analysis

Data from weight gain, food intake, food conversion efficiency, water consumption, nutrient digestibility, nitrogen retention and metabolisable energy content were subjected to analysis of variance using the SPSS/PC +V5.0 computer programm of Norusis (1992). Treatment means were tested for difference by use of the Least Significant Difference test (Snedecor and Cochran, 1980). All statements of significance are based on a probability of less than 0.05.

Results

Experiment 1.

Weight gain and food utilization of birds fed diets containing blended animal fat were respectively, 10.8 and 8.6% less favourable ($P < 0.05$) than in chicks fed on the soya oil-based diet (Table 3). The improvement in performance produced by enzyme supplementation depended on the type of fat added to the diets. Endo-xylanase added to the soya oil diet, had little effect on weight gain, whereas there was a (non-significant) 3% improvement in food utilisation ($P > 0.05$). When the blended animal fat diet was supplemented with an endo-xylanase, both weight gain and food utilisation were improved by approximately 9.5 and 6%, respectively ($P < 0.05$). Mortality was low, 1.7%, with no appreciable differences in mortality being observed among the treatment groups.

Experiment 2.

The water intake of birds fed on the diet containing animal fat was greater ($P < 0.05$) than in the birds fed on the soya oil diet (Table 4). The water/food ratio tended to increase when birds were fed on the blended animal fat diets ($P > 0.05$). Addition of an endo-xylanase to the diet containing 65 g soya oil/kg had little effect on water intake and water:food ratio ($P > 0.05$). When endo-xylanase was added to the diet containing blended animal fat, water intake was decreased ($P < 0.05$), whereas water:food ratio was little affected.

Table 3. *Effect of supplementation with an endo-xylanase preparation on chick performance in relation to the fat source; experiment 1 (1 to 21 d of age)*

Group	Fat source	Enzyme addition	Weight gain (g)	Food intake (g/bird/d)	Food/ gain ratio
I	soya oil	-	636^{b}	48.7	1.610^{ab}
II	soya oil	+	638^{b}	47.3	1.556^{a}
III	animal fat	-	567^{a}	47.2	1.748^{c}
IV	animal fat	+	621^{b}	48.6	1.644^{b}
SEM (df=20)			17.7	1.3	0.0205

[a,b,c] Mean values within a column with no common superscripts differ significantly ($P \leq 0.05$).

Digestibility of fat decraesed ($P < 0.05$) when soya oil was substituted by blended animal fat (Table 5); consequently the metabolisable energy value of the blended animal fat diet was lower ($P < 0.05$). NFE + crude fibre digestibility was greater in chicks fed on the blended animal fat diet than in those fed on the soya oil diet ($P < 0.05$). Nitrogen retention tended to decrease when soya oil was substituted by blended animal fat ($P > 0.05$). The effect of enzyme supplementation on digestibility depended on the type of fat added to the diet. Endo-xylanase added to in the diet containing 65 g soya oil/kg, had no significant effect on the parameters presented in Table 5 ($P > 0.05$), but there was a tendency towards improved fat digestibility ($P > 0.05$). Nitrogen retention was little affected when the soya oil diet was supplemented with endo-xylanase. Endo-xylanase added the blended animal fat diet increased the digestibilities of organic matter, crude fat and NFE + crude fibre significantly by 3.0%, 9.4% and 2.1%, respectively. As a consequence, metabolisable energy value and nitrogen retention increased when the blended animal fat diet was supplemented with the endo-xylanase preparation ($P < 0.05$).

Table 4. *Effect of supplementation with an endo-xylanase preparation on water consumption in relation to fat source; experiment 2*

Group	Fat source	Enzyme addition	Water intake (g/bird/d)[1]	Water/food ratio
I	soya oil	-	248^a	1.88
II	soya oil	+	245^a	1.88
III	animal fat	-	262^b	1.97
IV	animal fat	+	248^a	1.95
SEM (df=12)			4.4	0.043

[1] Means of 4 d.

[a,b] Mean values within a column with no common superscripts differ significantly ($P \leq 0.05$).

The digestibilities of amino acids tended to be greater (+ 2.1%) in birds fed on the soya-oil diet than in those fed on the blended animal fat diet (Table 6). The improvement in the digestibilities of amino acids after supplementation of the diets with the enzyme preparation was more pronounced in the diet containing blended animal fat than in the diet containing soya oil. These improvements in digestibility of the individual amino acids varied between -0.5% and +1.5% in the soya oil diet and between +0.9% and +4.3% in the blended animal fat diet. The average overall improvements in amino acid digestibility by enzyme addition was only 0.7% in the soya oil diet, but was 1.9% when blended animal fat was used as the main fat source.

Discussion

The results of the digestibility trial indicated that the difference observed in food:gain ratio between soya oil and blended animal fat in the absence of a xylanase was most likely to result from a difference in fat digestibility, because amino acid digestibility was not significantly affected. However, it should be noted that the performance data are not strictly comparable with the results of the digestibility trial. The performance data were obtained from broilers between 1 and 21 d of age, whereas the results for digestibility were obtained with 4 week-old chickens. It is well known that digestibility of fat, in particular animal fat, is influenced by age (Ketels and De Groote, 1988). In addition, the anti-nutritional effect of wheat is also more pronounced in young birds than in older birds (Veldman and Vahl, 1994). Furthermore, it should be noted that the results of the present study were obtained by using diets not supplemented with an antibiotic and a coccidiostat. Both additives might interfere with dietary xylanases. Support for an interaction with antibiotics is seen in the results of Elwinger and Säterby (1986), Elwinger and Teglöf (1991) and Schutte *et al.* (1994), which indicated that the effects of dietary xylanases and ß-glucanases were reduced by including an antibiotic in a wheat- or barley-based diet. There is no published information to indicate whether a coccidiostat will influence the effect of the dietary addition of xylanases.

The present study demonstrates that in chicks fed on diets containing wheat and rye, performance is affected by the fat source. Similar findings were observed by Antoniou and Marquardt (1982), who found that fat digestibility in rye-fed birds was depressed more when tallow rather than soya oil was the fat source in the diet. The present study clearly demonstrates that the effect of a dietary enzyme supplementation also depends on the type of added dietary fat. The improvement in performance and in the digestibility of nutrients, particularly of fat and AME value, after supplementation of the diets with enzymes, was more pronounced when diets contained blended animal fat than with soya oil.

The mechanism of the action of dietary enzymes is not yet clear. Increased viscosity is considered to be the most important mechanism by which pentosans reduce digestibility, through interference with the movement of digesta throughout the intestinal lumen (Antoniou *et al.*, 1981; Fengler and Marquardt, 1988). It is also suggested that the increase in viscosity may limit hydrolysis by enzymes and interfere with the transport of pancreatic enzymes and bile acids to the dietary nutrients. However, in the present study, an increase in digestibility of NFE + crude fibre and a marked reduction in nitrogen retention were also observed when blended animal fat was substituted for soya oil. This indicates that other mechanisms may also be involved.

Table 5. *Effect of supplementation with an endo-xylanase preparation on the digestibility, metabolisable energy content and nitrogen retention in relation to the fat source; experiment 2 (24 to 28 d of age)*

	Treatment				SEM
	I	II	III	IV	(df=11)
Added soya oil (g/kg)	65	65	5	5	
Added blended animal fat (g/kg)	-	-	60	60	
Enzyme addition	-	+	-	+	
Digestibility coefficients					
organic matter	0.665^{ab}	0.671^{ab}	0.658^{a}	0.688^{b}	0.57
crude fat	0.783^{c}	0.802^{c}	0.605^{a}	0.699^{b}	1.53
NFE + crude fibre[1]	0.709^{a}	0.713^{a}	0.736^{b}	0.757^{c}	0.50
ash	0.243	0.247	0.230	0.244	0.56
N retention[2] (% of intake)	50.2^{ab}	50.6^{b}	47.3^{a}	50.1^{b}	0.92
Metabolisable energy (ME_n, MJ/kg)	11.2^{b}	11.4^{b}	10.8^{a}	11.5^{b}	0.10
Dry matter content of droppings	0.267	0.259	0.258	0.250	-

[1] NFE: nitrogen-free extract (calculated as organic matter - crude protein - crude fat - crude fibre).

[2] Calculated as the differences between nitrogen intake and nitrogen excretion.

[a,b,c] Mean values within a row with no common superscripts differ significantly ($P \leq 0.05$).

Published results (Choct *et al.*, 1992) suggest that the increased flow of undigested nutrients to the lower part of the tract, as a result of the anti-nutritive effect of pentosans, promotes undesirable microbial growth and, consequently, increased microbial activity. Campbell *et al.* (1983) showed that in rye-fed birds the depression in weight gain and in fat retention were more pronounced in conventional chicks than in germ-free chicks. Introduction of *Streptococcus* to the germ free chicks produced a response similar to that obtained with conventional chicks. Furthermore, Wagner and Thomas (1978) attributed the depression in weight gain in rye-fed chicks to an increased microbial activity, particularly of spore-forming bacteria.

Bile acids are necessary for emulsification of fats, which is necessary for fat absorption. Young birds have a limited capacity to produce bile salts (Kussaibati *et al.*, 1982). In poultry, bile acids are recycled efficiently by reabsorption of bile acids from the intestinal tract (Hulan and Bird, 1972). A reduction in the recycling of bile acids causes a decrease in the size of the bile acid pool, reducing bile acid concentration in the intestinal digesta. This affects fat emulsification negatively, and consequently reduces fat absorption. The digestion of the long chain and saturated fatty acids would be affected to a greater extent than the soluble short chain and unsaturated fatty acids because the short chain and unsaturated fats (fatty acids) are more easily absorbed in the absence of bile acids (Garrett and Young, 1975). Studies by Kritchevsky (1978) have demonstrated that fibrous foods bind bile acids and thereby increase their excretion. The viscous pentosans might exert a similar effect on bile acids and increase bile acid excretion.

Many intestinal bacteria, including the genera *Streptococcus, Enterococcus, Bifidobacterium, Clostridium, Bacteroides,* and *Lactobacillus*, are known to catalyse deconjugation of bile acids (Coates, *et al.*, 1981; Musada, 1981; Chateau *et al.*, 1994; Grill *et al.*, 1995). From these findings, it can be hypothesised that the increased digesta viscosity and the increased microbial activity may cause a reduction in conjugated bile acid, which would affect fat emulsification negatively. As a consequence, digestibility of blended animal fat, containing mainly saturated fatty acids, would decrease to a greater extent than that of vegetable oil, containing high proportions of unsaturated fatty acids. To support this theory, Campbell *et al.* (1983) and Fengler *et al.* (1988) showed that supplementation of conjugated bile acids to rye-based diets improved animal fat digestibility.

Table 6. *Effect of supplementation with an endo-xylanase preparation in relation to fat source on the digestibility of amino acids; experiment 2 (24 to 28 d of age)*

	Treatment				SEM
	I	II	III	IV	(df=11)
Fat addition:					
soya oil (g/kg)	65	65	5	5	
blended animal fat (g/kg)	-	-	60	60	
Enzyme addition	-	+	-	+	
Isoleucine	0.761^{ab}	0.774^{b}	0.746^{a}	0.767^{ab}	0.79
Leucine	0.765	0.775	0.749	0.772	0.79
Lysine	0.794	0.794	0.773	0.787	0.63
Methionine	0.860^{ab}	0.866^{b}	0.843^{a}	0.860^{ab}	0.78
Cystine	0.597^{bc}	0.609^{c}	0.555^{a}	0.568^{ab}	1.19
Methionine + cystine	0.744^{b}	0.748^{b}	0.718^{a}	0.732^{ab}	0.67
Phenylalanine	0.703^{ab}	0.715^{b}	0.678^{a}	0.721^{b}	0.98
Threonine	0.677^{ab}	0.690^{b}	0.658^{a}	0.686^{b}	0.85
Valine	0.729^{ab}	0.737^{b}	0.710^{a}	0.731^{ab}	0.78
Arginine	0.803^{ab}	0.807^{b}	0.782^{a}	0.797^{ab}	0.70
Histidine	0.759	0.746	0.739	0.748	0.85
Alanine	0.724	0.726	0.706	0.720	0.86
Aspartic acid	0.690^{b}	0.696^{b}	0.667^{a}	0.688^{ab}	0.76
Glutamic acid	0.826	0.831	0.814	0.825	0.58
Glycine	0.665^{ab}	0.670^{b}	0.637^{a}	0.651^{ab}	0.97
Serine	0.708^{ab}	0.723^{b}	0.691^{a}	0.722^{b}	0.29
Mean amino acids	0.753	0.760	0.734	0.753	-

^{a,b,c} Mean values within a row with no common superscripts differ significantly ($P \leq 0.05$).

It is well documented that dietary enzymes can eliminate the anti-nutritional activity of pentosans (Annison, 1992; Schutte *et al.*, 1993). It has been shown that enzymes degrade pentosans which causes a reduction in digesta viscosity (Bedford and Classen, 1993). The latter might cause a reduction in microbial activity. This is supported by Choct *et al.* (1996), who showed that the addition of a xylanase based enzyme preparation to a diet containing wheat pentosans, reduced volatile fatty acid concentration in the ileal digesta. As a consequence, the concentration of conjugated bile acids and the digestibility of nutrients might be increased. This might explain the finding of the present study that the magnitude of the effect of an enzyme addition is much greater in diets containing animal fat than in diets containing vegetable oil.

In conclusion, this study has demonstrated that there is an interaction between fat source in wheat- and rye-based diets and endo-xylanase supplementation. The results clearly demonstrate that supplementation of a wheat-and rye-based diet containing a saturated fat with an endo-xylanase can overcome the malabsorption of the saturated fat. However, more information is needed to elucidate the mechanism of the anti-nutritional activity of pentosans from wheat and rye in relation to fat source, bile acids and intestinal microbial activity.

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References

Annison G. (1990) Polysaccharide composition of Australian wheats and the digestibility of their starches in broiler chicken diets. *Australian Journal of Experimental Agriculture* 30: 183-186.

Annison, G. (1992) Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology* 38: 105-121.

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

Antoniou, T., Marquardt, R.R. & Cansfield, P.S. (1981) Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agricultural Food and Chemistry* 8: 1240-1247.

AOAC (1984) Official Methods of Analysis, 14 th edn. (Washington DC, Association of Official Analytical Chemists).

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

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

1577.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens: effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. *British Poultry Science* 24: 191-203.

Chateau, N., Deschamps, A.M. & Hadj Sasi, A. (1994) Heterogeneity of bile salts resistance in *Lactobacillus* isolates of a probiotic consortium. *Letter Applied Microbiology* 18: 42-44.

Choct, M. & Annison, G. (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992) The inhibition of nutrient digestion by wheatpentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M., Annison, G. & Trimble R.P. (1992) Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition* 122: 2457-2465.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Coates, M.E., Cole, C.B., Fuller, R., Houghton, S.B. & Yokota, H. (1981) The gut microflora and the uptake of glucose from the small intestine of the chick. *British Poultry Science* 22: 289-294.

Elwinger, K. & Säterby, B. (1986) Comparison of beta-glucanase and virginiamycin in broiler diets containing barley and oats. *Proceedings of the 7th European Poultry Conference*, Paris, France, pp. 249-253.

Elwinger, K. & Teglöf, B. (1991) Performance of broiler chickens as influenced by a dietary enzyme complex with and without antibiotic supplementation. *Archiv für Geflügelkunde* 55: 69-73.

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

Fengler, A.I., Pawlik, J.R. & Marquardt, R.R. (1988) Improvement in nutrient retention and changes in excreta viscosities in chicks fed rye-containing diets supplemented with fungal enzymes, sodium taurocholate and penicillin. *Canadian Journal of Animal Science* 68: 483-491.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Grill, J.P., Manginot-Dürr, C., Schneider, F. & Ballongue, J. (1995) Bifidobacteria and probiotic effects: action of *bifidobacterium* species on conjugated bile salts. *Current Microbiology* 31: 23-27.

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

Hill, F.W. & Anderson, D.L. (1958) Comparison of metabolizable energy and productive energy determinations with growing chicks. *Journal of Nutrition* 64: 587-603.

Hulan, H.W. & Bird, F.H. (1972) Effect of fat level in isonitrogenous diets on the composition of avian pancreatic juice. *Journal of Nutrition* 102: 459-468.

Ketels, E. & De Groote, G. (1988) The nutritional value for broilers of fats characterized by short-chain fatty acids as affected by level of inclusion and age. *Animal Feed Science and Technology* 22: 105-118.

Kritchevsky, D. (1978) Influence of dietary fiber on bile acid metabolism. *Lipids* 13: 982-985.

Kussaibati, R., Guillaume, J., Leclercq, B. & Lafont, J.P. (1982) Effects of the intestinal microflora and added bile salts on the metabolisable energy and digestibility of saturated fats in the chicken. *Archiv* *für Geflügelkunde* 46: 42-46.

Masuda, N. (1981) Deconjugation of bile salts by *Bacteroides* and *Clostridium. Microbiology and Immunology* 25: 1-11.

National Rerearch Council (1994) Nutrient requirements of poultry, 9th revised edn. (Wasington DC, National Academy of Sciences).

Norusis, M.J. (1992) SPSS/PC+ Base System User's Guide Version 5.0 (SPSS inc., Chicago, Illinois).

Petterson, D. & Åman, P. (1988) Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. *Animal Feed Science and Technology* 20: 313-324.

Petterson, D. & Åman, P. (1989) Enzyme supplementation of a poultry diet containing rye and wheat. *British Journal of Nutrition* 62: 139-149.

Schutte, J.B., Geerse, C. & De Jong, J. (1993) Effect of enzyme supplementation to wheat-based diets on broiler chick performance. *Proceedings of the Symposium on Enzymes in Animal Production*, Kartause Ittingen, pp. 133-136.

Schutte, J.B., De Jong, J. & Borselli, E. (1994) Interaction effects of virginiamycin and a xylanase enzyme preparation on broiler performance. *Proceedings of the 9th European Poultry Conference*, Glasgow, UK, pp. 445-446.

Slump, P. (1969) Karakterisering van de voedingswaarde van eiwitten in voedingsmiddelen door hun aminozuursamenstelling en de invloed van verhitting en loogbehandeling op de benutbaarheid van aminozuren. *Ph.D. Thesis*, Free University Amsterdam, Amsterdam, The Netherlands.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical methods. 7th edn. (The Iowa State University Press. Ames, 1A).

Veldman, A. & Vahl, H.A. (1994). Xylanase in broiler diets with differences in characteristics and content of wheat. *British Poultry Science* 35: 537-550.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

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

Chapter 3

Nutritional implications of pectins in chicks in relation to esterification and origin of pectins

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Nutritional implications of pectins in chicks in relation to esterification and origin of pectins

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Abstract

An experiment was conducted to examine the effect of feeding high-methylated (HM) and low-methylated (LM) pectin on performance and physiological characteristics in broiler chicks. Two levels each (1.5 and 3%) of HM citrus pectin (HMC), LM citrus pectin (LMC), or HM sugar beet pectin (HMS) were added to a semi-purified basal diet. The experiment was conducted in battery brooders, and chicks received the diets as pellets from 6 to 27 d of age. The inclusion of LMC or HMS in the diet had little or no effect on the performance of chicks; however, when HMC was added to the diet growth and feed utilization were reduced significantly ($P < 0.05$). Water intake and water:feed ratio were increased ($P < 0.05$) after inclusion of all three pectin products in the diet ($P < 0.05$). The effect on water consumption was more pronounced in the HMC-containing diets than in the LMC or HMS diets, and was dose-dependent. *In vitro* viscosity was increased significantly by incorporating HMC or LMC into the diet, whereas HMS had no effect on this parameter. Water-holding capacity of the diets and the excreta were increased significantly by including one ($P < 0.05$) of the three pectin products in the diet. The concentration of some of volatile fatty acids (VFA) in the cecal chyme was markedly decreased ($P < 0.05$) by feeding HMC, whereas LMC had no effect ($P > 0.05$) on the concentration of VFA. In contrast, inclusion of HMS in the diet significantly increased the concentration of VFA in the cecal content ($P < 0.05$) dose-dependently. Based on the results of the present study, it can be concluded that the effect of dietary pectin on chick performance is dependent on the degree of carboxyl groups that is esterified, the origin of the pectin product, and the amount added to the diet.

Introduction

Pectins are important constituents of the primary cell walls and intercellular regions of higher plant tissues (e.g. soyabean oilmeal, rapeseed meal, and sunflower meal) and are extremely variable in chemical structure. This variation in structure of pectins affects the physical-chemical properties, which might affect nutritional-physiological activities. Pectin (or polygalacturonide) is a collective term for polysaccharides that are characterized by a linear backbone of 1→4 linked α-D-galacturonic acid units. These units are interrupted by L-rhamnose-rich regions, wich mainly contain L-arabinose, D-galactose- and L-xylose as side chains. The carboxyl groups of D-galacturonic acid are methylated to a different extent, and they may be acetylated. The main property of pectin is its ability to form a gel. Furthermore, pectins have a high water-binding capacity (Armstrong *et al.*, 1993) and can be easily fermented by the intestinal microflora (McBurney *et al.*, 1985).

In the human food and pharmaceutical industry, much research is done with pectins regarding the influence of the structure on gel formation. The degree of esterification, a measure of the percentage of carboxyl groups esterified with methanol, plays a major role in the gel-forming capacity and gel strength (Pilnik and Voragen, 1970; BeMiller, 1986; May, 1990). Pectins in which the percentage of esterified carboxyl groups is higher than 50% are high-methylated (HM), whereas a percentage of esterification lower than 50% defines the low-methylated (LM) pectins. An HM pectin easily forms a gel with sugar and acid. An LM pectin forms a gel in the presence of cations such as calcium, regardless the pH. When a pectin is acetylated, gel formation will be hampered or even blocked.

The degree of esterification of pectins can affect the performance of rats (Judd and Truswell, 1982). HM pectin in the diet has stronger depressing effects on performance and plasma cholesterol levels than LM pectin; however, Drochner *et al.* (1990) suggested that the effect of pectins on layer hen performance is independent of the degree of esterification of pectin. Literature studies regarding the effect of pectins on broiler chicks have been based on HM citric pectin only (Vohra and Kratzer, 1964; Wagner and Thomas, 1977; Patel *et al.*, 1980, 1981; Bishawi and McGinnis, 1984). These studies showed a clear depressing effect on growth and feed utilization after addition of 3 to 4% HM citrus pectin (HMC) to the diet (Vohra and Kratzer, 1964; Wagner and Thomas, 1977; Patel *et al.*, 1980, 1981; Bishawi and McGinnis, 1984). These negative effects on broiler performance could be partly explained by reduced feed intake (Vohra and Kratzer, 1964; Patel *et al.*, 1981; Bishawi and McGinnis, 1984). Furthermore, it was reported that sticky droppings occurred (Wagner and Thomas, 1977; Patel *et al.*, 1980). Bishawi and McGinnis (1984) also showed an increase in fecal viscosity, a lower percentage of carcass fat, and a lower liver weight at an inclusion level of 4% HMC in the diet. The latter could not be confirmed by Patel *et al.* (1981). Wagner and Thomas (1978) reported an increase in volatile fatty acid (VFA) production in the cecal content after addition of 4.5% HMC to the diet, indicating an increase in fermentation of the microflora. Knowledge on whether the degree of esterification has an influence on broiler performance is not available. A better understanding of the effects that the different pectins have on poultry production is needed in view of the trend to the use of a wide variety of byproducts in the poultry industry. The purpose of the current research was to study the effects of two different dietary levels of HM and LM pectin on performance, water intake, and some characteristics in the chyme in broiler chicks in relation to the origin of the pectin product.

Materials and methods

Experimental design

A semi-purified basal diet was used to insure that it was pectin-free (Table 1). The diet was calculated to be adequate in all nutrients according to the National Research Council (1994). There were seven treatment groups. The control diet contained 5% wood cellulose. The experimental diets were supplemented with 1.5 and 3% of HM citrus pectin (HMC; Citrus pectin, type CU 301, methoxylation > 65%, contined b.v., The Netherlands), LM citrus pectin (LMC; Citrus pectin, type CU 12, methoxylation 30 to 38%, contined b.v., The Netherlands) or HM sugar beet pectin (HMS; Citrus pectin, type RU 301, methoxylation > 60%, contined b.v., The Netherlands), respectively. The pectin products, supplied as anhydrous poly-saccharides, were substituted by weight for wood cellulose. The diets were consumed *ad libitum* as pellets. Water was also available for *ad libitum* consumption.

One-day-old female Ross broiler chicks were used. The birds were housed in three tiered, electrically heated battery cages with a floor space of 0.975 m^2 and wire floors. The cages were situated in an insulated room with facilities to control temperature and humidity. Chicks were subjected to continuous artificial fluorescent illumination. A standard practical diet was fed for the first 6 d. At 6 d of age, 15 birds were allotted to each of 42 cages such that the average body weight (126 g) and weight range (110 to 150 g) were similar. Each treatment group was randomly allotted to six cages each. The experimental diets were fed for a period of 21 d (from 6 to 27 d of age).

At the end of the trial, chicks were weighed individually, and feed consumption of each cage was recorded. During the last 12 d of the experimental period, water consumption was measured for each cage over a 12 h period. Water intake was measured as the difference in the volume of water in the pans at the beginning and end of the treatment period, without correction for evaporative water loss. In order to determine the water:feed ratio, feed intake was also recorded during the water intake measurements.

Table 1. *Composition of the basal diet*

Ingredient	Percentage
Corn	40
Meritose (glucose)	19.85
Soya oil	1.3
Animal fat	2.0
Isolated soya protein (88% CP)	8.1
Casein	5.4
Corn gluten meal	5.0
Feather meal, hydrolized	1.5
Meat meal tankage (58% CP)	5.0
Molasses	2.0
Cellulose (Arbocal B 800)	5.0
Limestone	0.88
Monocalcium phosphate	1.05
Vitamin + mineral mix[1]	1.0
L-lysine HCL	0.14
DL-methionine	0.17
L-threonine	0.03
L-tryptophan	0.02
L-arginine	0.26
KCL	0.10
$KHCO_3$	1.2
Calculated contents:	
Crude protein (%)	23.1
Metabolizable energy (kcal/kg)[2]	3020
Ca (%)	0.85
P (%)	0.62
Lysine (%)	1.22
Methionine + cystine (%)	0.93

[1] Supplied per kg of diet: vitamin A (retinol acetate), 10,000 IU; cholecholciterol, 2,000 IU; vitamin E (DL-α-tocopherol acetate), 20 IU; menadione, 5 mg; riboflavin, 4 mg; D-pantothenic acid, 12 mg; niacinamide, 40 mg; cobalamin, 0.015 mg; choline chloride, 500 mg; biotin, 0.1 mg; folic acid, 1 mg; Fe, 60 mg; Mn, 60 mg; Cu, 25 mg; Zn, 100 mg; Se, 0.05 mg; I, 3.5 mg; anti-oxidant, 100 mg; virginiamycin, 20 mg.

[2] Calculated from the data provided by the Dutch Livestock Committee.

During the last day of the experimental period, excreta from four cages per treatment group were collected quantitatively from plastic trays into containers at intervals of 4 h, and stored at -18 C. Immediately after the collection period the excreta were homogenized (without the addition of water), sampled, and freeze-dried for the determination of water-holding capacity (WHC).

Feed and water were withheld for 0.5 h after the termination of the trial, and 112 birds were then killed by injection of T 61 (Embutramide-Mebezoniumiodide-Tetracainhydrochloride mix Hoechst, München D-87516, G). Sixteen representable birds per treatment were selected. Body weight, weight of the small intestine, cecal weight including its contents, and liver weight without gall bladder were measured individually. A pooled sample of digesta from the ceca of four chicks per cage was taken for VFA analysis.

Chemical Analysis

Concentration of VFA in cecal content was determined according to the gas-liquid chromatographic method (GLC; Model HP 5890, Hewlett Co., Palo Alto, CA 94304) of Schutte *et al.* (1991). A known portion (about 5 g) of the cecal content was immediately acidified after collection with 500 µL phosphoric acid (850 mL/L), and 3 mL of an aqueous solution of isocapronic acid (4.0193 g/L) was added as an internal standard. Distilled water was added to the mixture to obtain a final volume of 10 mL. A 1 µL sample of the final solution was injected into the GLC.

Three samples of the experimental diets, and fresh samples of the excreta were freeze-dried for measuring the WHC according to the method of Armstrong *et al.* (1993). One g of the freeze-dried samples was hydrated overnight in a solution of sodium-azide (825 ml), and then placed into dialysis tubes (Size 4 - 22/32 Spectrum Medical Ind. Inc., Los Angelos, CA 90060). The dialysis tubes were placed in a 100/150 mL conical flask containing 50 mL polyethylene glycol solution (PEG) (PEG 10,000 MW, BDH Chemicals, Poole, Dorset, BH12 4NN, UK) to measure the WHC.

The viscous properties of the experimental diets were determined with a Brookfield digital viscometer (Model LVTD VCP-II, Brookfield Engineering Laboratories, Inc., Stoughton, MA 02072), according to the method of Bedford and Classen (1993). *In vitro* viscosity of three samples was determined by using the following assay conditions; digestion for 45 min with pepsin (2,000 U/mL)/0.1 *N* HCl, followed by digestion for 120 min in 2 mg/mL pancreatin in 1 *M* $NaHCO_3$.

Statistical Analysis

The data were subjected to analysis of variance using the SPSS/PC + V5.0 computer program of Norusis (1992). The treatment factors were type of diet and battery tier using the following model:

$$y = \mu + a.G + b.T + e,$$

where y = the response measurements; G = treatment group, and T = tier of the battery.

Treatment means were tested for difference by use of the Least Significant Difference test (Snedecor and Cochran, 1980). All statements of significance are based on a probability of less than 0.05.

Table 2. *The effect of the inclusion into the diet of two levels of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC), and high-methylated sugar beet pectin (HMS) on the performance of chicks from 6 to 27 d of age*

Diet	Pectin level (%)	Weight gain (g)	Feed intake (g/bird/d)	Feed:gain (g:g)	Water intake (g/bird/d)[1]	Water: feed (g:g)
Control		1,027^{d}	76.8^{b}	1.571^{a}	158^{a}	1.64^{a}
HMC	1.5	982^{b}	77.8^{b}	1.653^{c}	182^{d}	1.88^{c}
HMC	3	734^{a}	70.1^{a}	2.009^{d}	179^{d}	2.11^{d}
LMC	1.5	1,021^{d}	77.0^{b}	1.584^{ab}	159^{ab}	1.65^{a}
LMC	3	1,019^{cd}	77.3^{b}	1.592^{ab}	175^{cd}	1.81^{bc}
HMS	1.5	994^{bc}	75.5^{b}	1.595^{ab}	154^{a}	1.64^{a}
HMS	3	1,005^{bcd}	76.8^{b}	1.604^{b}	167^{bc}	1.74^{b}
SEM (df=33)		9.2	0.78	0.0105	3	0.02

$^{a-d}$ Mean values within a column with no common superscript differ significantly ($P \leq 0.05$).

[1] Means of three periods of 4 d.

Results

The addition of 1.5 and 3% HMC to the diet decreased ($P < 0.05$) weight gain and feed utilization (Table 2). The HMC at 3% compared to 1.5% further depressed growth and efficiency of feed utilization by approximately 30% ($P < 0.05$). In addition, daily feed intake was decreased ($P < 0.05$) after the addition of 3% HMC to the diet. Moreover, HMC increased water intake and water:feed ratio ($P < 0.05$). The effect of HMC on the water:feed ratio was dose-dependent as it was increased by 15 and 30%, respectively, by the inclusion of 1.5 and 3% HMC into the basal diet. Using LMC in the diet did not affect weight gain, feed conversion efficiency, or feed intake ($P > 0.05$). Water intake and the water:feed ratio were also not affected at an inclusion level of 1.5% LMC in the diet ($P > 0.05$). However, at an inclusion level of 3% LMC in the diet, the water intake and the water:feed ratio were 10% higher than the control group. Inclusion of 1.5% HMS in the diet significantly depressed weight gain. Growth was also depressed after the addition of 3% HMS to the diet. The decrease of feed utilization when 3% HMS was added to the diet was 2.1% ($P < 0.05$). Water intake and water:feed ratio were not affected after an addition of 1.5% HMS to the diet ($P > 0.05$); however, inclusion of 3% HMS in the diet increased water intake and water:feed ratio by approximately 6% ($P < 0.05$). Mortality rate was very low (1.1%) with no appreciable differences in mortality being observed among the treatment groups.

The inclusion of HMC and LMC, but not HMS, in the diet increased ($P < 0.05$) the *in vitro* viscosity of the diets (Table 3). When included in the diets, HMC, LMC or HMS significantly increased (on average 42%) the WHC of the diets. Similarly, the WHC of the excreta was increased when all three pectin products were added to the diets, with the exception of the 1.5% HMS treatment group. The effect of LMC on WHC of the excreta at a dietary level of 3% was significantly higher than that of HMC or HMS.

Table 3. *The effect of the inclusion into the diet of two levels of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC), and high-methylated sugar beet pectin (HMS) on its viscosity and water-holding capacity (WHC) and the WHC of excreta*

	Pectin	Viscosity	WHC (g:g)	
Diet	level (%)	diets (kPa)	diets	excreta
Control		2.08^{ab}	0.79^{a}	1.17^{a}
HMC	1.5	2.97^{c}	1.05^{c}	1.32^{b}
HMC	3	8.07^{e}	1.14^{e}	1.43^{c}
LMC	1.5	5.31^{d}	1.02^{bc}	1.32^{b}
LMC	3	7.86^{e}	1.06^{cd}	1.65^{d}
HMS	1.5	1.87^{a}	0.97^{b}	1.16^{a}
HMS	3	2.47^{b}	1.11^{de}	1.31^{b}
SEM		0.16	0.02	0.03
df		14	14	20

[a-e] Mean values within a column with no common superscript differ significantly ($P \leq 0.05$).

Data regarding liver, small intestine, and cecal weights are shown in Table 4. Inclusion of HMC, LMC, or HMS in the diet tended to reduce relative liver weight. The relative weight of the small intestine was only affected by inclusion HMC in the diet. The addition of 1.5 and 3% HMC to the diet increased intestinal weight by 8.9% and 21.1%, respectively. Cecal weight was not affected by addition of LMC to the diet, or low levels (1.5%) of HMC or HMS; however, cecal weights for chicks fed the diets that contained 3% HMC or HMS were 40 and 25% larger, respectively, than for chicks fed the control diet.

The concentration of total VFA in the cecal contents tended to decrease at an inclusion level of 3% HMC in the diet (Table 5). This reduction was significant for propionic acid, butyric acid, and valeric acid. The LMC had no influence on the concentration of total VFA or individual fatty acids. Birds fed HMS, in contrast to the other groups, had increased VFA concentrations in the ceca. The increase in VFA concentration for the 3% HMS group compared to the control group was significant. This effect on VFA was mainly caused by an increase in concentration of acetic acid and butyric acid.

Discussion

The present study indicated that the effect of dietary pectin on the performance of broiler chicks are strongly influenced by the degree of esterification or the origin of the pectin product, and the amounts that are added to the diet. Addition of HMC to the diet strongly depressed performance and increased water consumption. These results are in agreement with the findings of other investigators (Vohra and Kratzer, 1964; Wagner and Thomas, 1977; Patel *et al.*, 1980, 1981; Bishawi and McGinnis, 1984). Several investigators suggested that the viscous property of pectin is the major factor responsible for its anti-nutritive effect (Patel *et al.*, 1980; Bishawi and McGinnis, 1984). The viscous properties of ß-glucans in barley (White *et al.*, 1981; Campbell *et al.*, 1989) and arabinoxylans (pentosans) in rye (Antoniou *et al.*, 1981; Bedford *et al.*, 1991; Bedford and Classen, 1992) or wheat (Choct and Annison, 1990; 1992) have also lead to the hypothesis that they are the primary cause of the anti-nutritive effects of nonstarch polysaccharides (NSP) in poultry. The marked increase in the viscosity of the diet that was obtained with HMC indicates that the anti-nutritional effects of HMC may be caused by its viscous properties.

Table 4. *The effect of the inclusion into the diet of two levels of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC), and high-methylated sugar beet pectin (HMS) on liver, small intestine, and cecal weights of chicks*

	Control	HMC		LMC		HMS		SEM
	--	1.5 % pectin	3 % pectin	1.5 % pectin	3 % pectin	1.5 % pectin	3 % pectin	(df=20)
Live weight (LW) at necropsy (g)	1,222^{b}	1,188^{b}	1,034^{a}	1,237^{b}	1,208^{b}	1,183^{b}	1,242^{b}	27
Liver weight (g)	48.5^{c}	42.9^{ab}	38.9^{a}	46.4^{bc}	46.1^{bc}	43.6^{abc}	45.9^{bc}	1.90
% of LW	3.97	3.61	3.76	3.75	3.83	3.70	3.70^{a}	0.12
Small intestine weight (g)	105.0^{a}	111.2^{a}	106.9^{a}	104.6^{a}	105.7^{a}	105.6^{a}	114.1^{a}	4.20
% of LW	8.58^{ab}	9.34^{b}	10.39^{c}	8.45^{a}	8.76^{ab}	8.93^{ab}	9.18^{ab}	0.30
Cecal weight (g)	8.6^{ab}	10.0^{bc}	10.1^{bc}	10.4^{bc}	7.9^{a}	8.8^{abc}	10.7^{c}	0.70
% of LW	0.69^{ab}	0.84^{bcd}	0.98^{d}	0.84^{bcd}	0.66^{a}	0.75^{abc}	0.87^{cd}	0.06

$^{a\text{-}d}$ Mean values within a row with no common superscript differ significantly ($P \leq 0.05$).

Romruen (1988) reported a negative effect on laying hen performance when 4 and 6% HMC were added to the diet. Similar negative effects on laying hen performance with 6% LMC in the diet were reported by Drochner *et al.* (1990). Drochner *et al.* (1990) concluded on basis of their results that both LM and HM pectin have comparable depressing effects on laying hen performance. This conclusion, however, may not be correct as our data strongly indicates that LMC in the diet do not affect chick performance. On the other hand, it is conceivable that the inclusion of 6% LMC into our diet would have also caused decrease in chick performance.

It has been previously reported by Bedford and Classen (1993) that an *in vitro* viscosity assay could be used to accurately predict the *in vivo* intestinal viscosity, and consequently predict the anti-nutritive activity of viscous compounds. This conclusion, however, is not supported by our study as there was little relationship in our study between the viscosity of the diet and the anti-nutritive properties of the pectin products that were used. The differences observed between the two research groups may, in part, be attributed to the nature of the viscous compounds that were used. The pectins used in the current study may have been more readily hydrolyzed by the intestinal microorganisms, than the pentosans present in rye in the study by Bedford and Classen (1993). Therefore, *in vitro* viscosity assays of the diet may not be good predictors of the intestinal viscosity. Therefore, in future measurements, the intestinal viscosity may provide additional information with regard to the effects of pectins in broiler chicks.

Results of the present study showed that addition of HMS to the diet did depress performance, but to a lower extent than that of HMC. Carboxyl groups present in sugar beet pectin are both methylated and acetylated (May, 1990), whereas those from citrus fruit are only methylated. Furthermore, the molecular weight of sugar beet pectin is lower than that of citrus pectin. Esterification with an acetyl group compared to a methyl group (BeMiller, 1986) and a lower molecular weight (Christensen, 1954) reduces the gel forming capacity of a pectin. The *in vitro* viscosity of the diets containing HMS was not higher than that of the control diet. This finding suggests that HMS has limited viscous capacity, which, consequently, reduces its ability to increase the viscosity of digesta in the small intestine.

Viscosity is considered to be a mechanism by which viscous NSP reduces digestibility through interference with the movement of digesta and pancreas enzymes across the intestinal lumen. It is suggested that the increased flow of undigested nutrients to the lower gut, as a result of the increased digesta viscosity, stimulates bacterial activity. In the present study, no increase in the concentration of VFA in the cecal content was found when HMC or LMC was added to the diet; however, inclusion

Table 5. *The effect of the inclusion into the diet of two levels of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC), and high-methylated sugar beet pectin (HMS) on the concentration of volatile fatty acids (VFA) in the cecal contents of chicks*

	Control	HMC		LMC		HMS		SEM
Fatty acid (g/L)	--	1.5 % Pectin	3 % Pectin	1.5 % Pectin	3 % Pectin	1.5 % Pectin	3 % Pectin	(df=20)
Total VFA	4.82^{ab}	4.65^{a}	4.36^{a}	4.60^{a}	5.17^{ab}	5.92^{b}	7.75^{c}	0.41
Acetic acid	3.00^{a}	3.29^{ab}	3.14^{a}	2.89^{a}	3.40^{ab}	3.98^{b}	5.63^{c}	0.28
Propionic acid	0.45^{b}	0.38^{ab}	0.32^{a}	0.41^{b}	0.44^{b}	0.45^{b}	0.40^{ab}	0.03
Butyric acid	0.92^{bc}	0.65^{ab}	0.58^{a}	0.91^{bc}	0.97^{c}	1.10^{cd}	1.30^{d}	0.10
Iso-butyric acid	0.07^{a}	0.06^{a}	0.06^{a}	0.07^{a}	0.06^{a}	0.06^{a}	0.05^{a}	0.01
Valeric acid	0.34^{bc}	0.22^{ab}	0.20^{a}	0.28^{abc}	0.26^{abc}	0.29^{abc}	0.36^{c}	0.05
Iso-valeric acid	0.06^{bc}	0.06^{bc}	0.07^{c}	0.05^{bc}	0.04^{ab}	0.05^{bc}	0.02^{a}	0.01

$^{a-c}$ Mean values within a row with no common superscript differ significantly ($P \leq 0.05$).

of 3% HMS in the diet increased cecal weight and VFA concentrations in the cecal content. Wagner and Thomas (1978) reported an increase in the activity of the microflora in the ileum in broiler chicks when pectin was added to the diet. Recently, Carré and Gomez (1994) showed an increase in VFA excretion in the feces of cecectomized adult roosters after addition of a pectin-based gelling agent to the diet. Therefore, pectins in the diet may affet the bacterial activity either in the small intestine or in the ceca depending on the origin of the product.

In the present study, all three pectin products increased WHC of the diets and the excreta. The capacity of a pectin to bind water can, at least in part, explain the increase in water:feed ratio of broiler chicks in the present study. The results of the present study are in agreement with the findings of Armstrong *et al.* (1993). These investigators reported in an experiment with rats that the addition of 10% HMC to the diet increased the WHC in both the diet and the excreta. Chemical and physical factors in the intestinal tract, and the microbial activity in the intestine affects the WHC of the excreta (Armstrong *et al.*, 1990). However, the possible effects these factors have on the WHC of the excreta remain unknown.

Based on the results of the present study, it can be concluded that the effect of dietary pectin on performance of broiler chicks strongly depends on its dietary level, the degree that it is esterified, and the origin of the pectin product. The data from the present study are insufficient to explain the differences found on performance between the pectin products. Before dietary pectinases can be used commercially, further work will be required to establish the mechanism which characterizes the differences among pectins.

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References

Antoniou, T., Marquardt, R.R. & Cansfield, E. (1981) Isolation, partial characterization and anti-nutritional activity of a factor (pentosans) in rye grain. *Journal of Agriculture and Food Chemistry* 29: 1240-1247.

Armstrong, E.A., Brydon, W.G. & Eastwood, M. (1990) Fiber metabolism and colonic water. In: *Dietary*

Fiber. (Kritchevsky, D., Bonfield, C. & Anderson, W.J., eds.) Plenum Press, New York, NY, pp. 179-186.

Armstrong, E.F., Eastwood, M.A. & Brydon, W.G. (1993) The influence of wheat bran and pectin on the distribution of water in rat caecal contents and faeces. *British Journal of Nutrition* 69: 913-920.

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

Bedford, M.R. & Classen, H.L. (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 the results in improved growth rate and food conversion efficiency of broiler chicks. *Journal of Nutrition* 122: 560-569.

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

Bemiller, J.N. (1986) An introduction to pectin: structure and properties. In: *Chemistry and Function of Pectin.* (Fishman, M.L. & Jen, J.J. eds.) American Chemical Society, Symposium Series 310, Publishing Co., New York, NY, pp. 2-12.

Bishawi, K.O. & McGinnis, J. (1984) Studies on the action of pectin in depressing the growth of chicks. *British Poultry Science* 25: 519-528.

Campbell, G.L., Rossnagel, B.F., Classen, H.L. & Thacker, P.A. (1989) Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. *Animal Feed Science and Technology* 26: 221-230.

Carré, B. & Gomez, J. (1994) Digestibility of water-soluble pectin and organic acid losses in intact or cecectomized adult roosters. *Poultry Science* 73: 1881-1886.

Choct, M. & Annison, G. (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Christensen, P.E. (1954) Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *Food Research* 19: 163-172.

Drochner, W., Ceroi, I.H., Stadermann, B. & Lüders, H. (1990) Einfluss steigender Zulagen niedrigveresterten Pektins in der Diät auf den Stoffwechsel von Legehennen - geprüft mit Hilfe von "Pair-Feeding-Versuchen". *Archive für Animal Nutrition* 40: 431-442.

Judd, P.A. & Truswell, A.S. (1982) Comparison of the effects of high- and low-methoxyl pectin on blood and faecal lipids in man. *British Journal of Nutrition* 48: 451-458.

May, M.B. (1990) Industrial pectin: sources, production and applications. Carbohydrate *Polymers* 12: 79-99.

McBurney, M.I., Horvath, P.J., Jeraci, L.J. & Van Soest, P.J. (1985) Effect of *in vitro* fermentation using human faecal inoculum on the water-holding capacity of dietary fibre. *British Journal of Nutrition* 53: 17-24.

National Research Council (1994) Nutrient Requirements of Poultry, 9th rev. ed. National Academy Press, Washington DC.

Norusis, M.J. (1992) SPSS/PC+ Base System User's Guide Version 5.0 (SPSS inc., Chicago, IL.

Patel, M.B., McGinnis, J. & Pubold, M.H. (1981) Effect of dietary cereal grain, citrus pectin, and guar gum on liver fat in laying hens and young chicks. *Poultry Science* 60: 631-636.

Patel, M.B., Jami, M.S. & McGinnis, J. (1980) Effect of gamma irritation, penicillin, and/or pectic enzyme on chick growth depression and fecal stickiness caused by rye, citrus pectin, and guar gum. *Poultry Science* 59: 2105-2110.

Pilnik, W. & Voragen, A.G.J. (1970) Pectic substances and other uronides. In: *Biochemistry of Fruits and their Products.* (Hulme, A.A., ed.) Academic Press, London, UK, pp. 53-87.

Romruen, K., Drochner, W., Lüders, H. & Günther, K.D. (1988) Auswirkungen steigender Pektinangaben an Legehennen auf Futterverträglichkeit, Verdaulichkeit der Nährstoffe und einige Parameter im Darmchymus und Serum, geprüft im "Pair-Feeding-Verfahren". *Journal of Animal Physiology and Animal Nutrition* 60: 257-268.

Schutte, J.B., De Jong, J., Polziehn, R. & Verstegen, M.W.A. (1991) Nutritional implications of D-xylose in pigs. *British Journal of Nutrition* 66: 83-93.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical Methods. 7th ed. The Iowa State University Press. Ames, 1A.

Wagner, D.D. & Thomas, O.P. (1977) A rye type growth depression of chicks fed pectin. *Poultry Science* 56: 615-619.

Wagner, D. D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Martlett, J.A. (1981) The viscosity interaction of barley ß-glucan with *Trichoderma viride* cellulase in the chick intestine. *Poultry Science* 62: 853-862.

Vohra, P. & Kratzer, F.H. (1964) Growth inhibitory effect of certain polysaccharides for chickens. *Poultry Science* 43: 1164-1170.

Chapter 4

Effect of dietary high- and low-methylated citrus pectin on activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks

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Abstract

A study was conducted with broiler chicks to evaluate the effects of dietary high-methylated citrus pectin (HMC) or low-methylated citrus pectin (LMC) on performance, nutrient digestibility, morphology of the small intestinal wall and ileal microbial activity. Both pectin products were tested at a dietary level of 30 g/kg by using a basal diet based on corn and soya flour. Inclusion of HMC in the diet, depressed weight gain and food utilization significantly. With a dietary addition of LMC there was only a small decrease in weight gain and food utilization. Digestibility of dry matter, organic matter, crude fat, starch and amino acids, nitrogen retention and metabolizable energy value of the diet were reduced significantly when HMC was added to the diet. The addition of LMC to the diet reduced fat and ash digestibility and metabolizable energy value of the diet significantly. Inclusion of LMC in the diet increased ileal viscosity marginally, whilst HMC had such an effect that the supernatant could not be extracted. Microbial activity in the ileum, particularly that of *Enterococci*, *Bacteroidaceae*, *Clostridia* and *E. coli*, was increased significantly with dietary addition of HMC. Inclusion of LMC in the diet did not greatly affect microbial activity as only the number of *Clostridia* was increased. The addition of HMC to the diet markedly affected morphology of the intestinal wall and significantly increased the number of goblet cells per 100 villus cells and the sucrase isomaltase activity were increased significantly. However, the morphology of the intestinal wall was hardly affected by LMC, whereas the number of goblet cells per 100 villi cells was significantly increased. Results of the present study indicate that inclusion of water-soluble pectins in the diets of chicks changes ileal microbial activity and morphology and histology of the small intestinal wall. The magnitude of these changes depends on the degree of methylation of the pectins.

Introduction

The inclusion level of many cereals, including barley, wheat and rye in diets of chicks is limited because of the presence of non-starch polysaccharides (NSP), which have anti-nutritive properties. These NSP, which relate to ß-glucans (barley) and arabinoxylans (pentosans; wheat and rye), interfere with digestion and absorption of nutrients especially in chicks. As a result, the performance of chicks is reduced. Numerous researchers have demonstrated that the soluble fraction of these NSP is mainly responsible for the anti-nutritive responses (Antoniou *et al.*, 1981; Fincher and Stone, 1986; Hesselman and Åman, 1986; Ward and Marquardt, 1987; Classen *et al.*, 1988; Choct and Annison, 1990; 1992). These water-soluble NSP can bind large amounts of water, and increase the viscosity of the aqueous fraction in the small intestine.

It is considered that this increase in viscosity is the primary mechanism by which these water-soluble NSP express their anti-nutritive properties (Choct and Annison, 1990, 1992; Bedford and Classen, 1993). Several studies indicate that the microflora also might play an important role in the anti-nutritive properties of water-soluble NSP. Wagner and Thomas (1978) showed an increase in anaerobic bacteria in the small intestine of chicks when rye or citrus pectin were included in the diet. These investigators suggested that the increase in microbial activity in the ileum might indirectly be responsible for the anti-nutritive effects of rye and citrus pectin. Hofshagen and Kaldhusdal (1992) reported that the number of Clostridia increased in the small intestine when barley was included in a wheat and oat based diet. Campbell *et al.* (1983) showed that the decrease in performance in rye-fed birds was more pronounced in conventional chicks than in germ-free chicks suggesting that the microflora enhanced with the anti-nutritive properties of the water-soluble NSP.

Sakata (1987) observed an increased gut secretion and a change in the morphology of the gut wall when the bacterial activity was increased in the gastrointestinal tract. A change of the mucosa of the small intestine of chicks was also observed by including barley or rye in the diet (Viveros *et al.*, 1994; Silba and Smithard, 1996). Viveros *et al.* (1994) showed that villi were shorter and thicker, and that the number of goblet cells was increased in the barley-fed birds. Smits (1996), however, showed that inclusion of viscous carboxymethylcellulose (CMC) to a broiler diet hardly affected gut morphology. Based on a study with rats, Gee *et al.* (1996) concluded that the effect of viscous carbohydrates on morphology is depended on the fermentability of the carbohydrate source. Recently, Langhout and Schutte (1996) showed that inclusion of HMC in a semi-synthetic diet markedly reduced broiler chick performance, whereas LMC only slightly reduced performance.

In the present study, high- (HMC) and low-methylated citrus pectin (LMC) were used as model carbohydrates to determine if the effects of the viscous, fermentable carbohydrates were associated with an effect on the microflora in the small intestine and/or with a change in gut morphology. The study involved a growth performance trial and a digestibility trial.

Materials and methods

Experiment 1: Growth performance trial

This experiment was designed to compare the effect of dietary high-methylated citrus pectin and low-methylated citrus pectin on growth, food intake and food conversion efficiency in broiler chicks. In addition, ileal viscosity, pH, morphology of the wall of the small intestine and composition of the microflora in the small intestine were determined. The reference basal diet contained corn and soya flour (Table 1). The basal diet was calculated to be adequate in all nutrients according to the National Research Council (1994). Additives such as antibiotics and coccidiostats, which may interfere with the microbial activity, were omitted from the diet. To the basal diet 30 g high-methylated citrus pectin/kg (HMC; type CU 301, methoxylation > 65%, Contined b.v., The Netherlands) or 30 g low-methylated citrus pectin/kg (LMC; type CU 12, methoxylation 30 to 38%, Contined b.v., The Netherlands) were added. The pectin products were added to the diets as anhydrous polysaccharides at the expense of wood cellulose. The ME value of wood cellulose and pectin was assumed to be zero.

One-day-old male Ross hybrid broiler chicks were used. The birds were housed in three tiered, electrically heated battery cages having wire floors with a floor space of 975 cm^2. The cages were located in an insulated room with controlled temperature and humidity. Chicks were subjected to continuous artificial fluorescent illumination. Each treatment group was allotted at random to 6 cages with 24 birds each. The experimental diets were fed for a period of 21 d (1 to 21 d of age). At the end of the trial, chicks were weighed individually. Food consumption and efficiency of food utilization were determined for each cage.

Table 1. *Composition of the basal diet in g/kg.*

Ingredient	
Maize	450
Tapioca	43.1
Soya oil	65.0
Soyaflour (500 g/kg CP)	230.0
Maize gluten meal	50.0
Fish meal (706 g/kg CP)	10.0
Meat meal tankage (580 g/kg CP)	40.0
Cellulose (Arbocel B 800)	65.0
Molasses	10.0
Limestone	9.2
Mono calcium phosphate	11.2
Salt	2.5
Vitamin + minerals[1]	10.0
L-lysine hydrochloride	2.2
DL-methionine	1.8
Contents:	
Dry matter (analysed, g/kg)	918
Crude protein (analysed, g/kg)	217
Crude fat (analysed, g/kg)	99
Ash (analysed, g/kg)	56
Starch (analysed, g/kg)	335
Metabolizable energy (calculated, MJ/kg)[2]	12.2
Ca (calculated, g/kg)	8.7
P (calculated, g/kg)	6.5
Lysine (analysed, g/kg)	12.8
Methionine + cystine (analysed, g/kg)	9.5

[1] Supplied per kilogram of diet: riboflavin, 4 mg; niacin amide, 40 mg; d-pantothenic acid, 12 mg; choline chloride, 500 mg; cyano cobalamin, 15 µg; Dl-α-tocopheryl acetate, 20 mg; menadione, 5 mg; retinyl acetate, 3.44 mg; cholecalciferol, 50 mg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.7H_2O$, 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 100 mg; $ZnSO_4$.H2O, 150 mg; $Na_2SeO_3.5H_2O$, 0.15 mg; KJ, 5 mg; $CoSO_4.7H_2O$, 1 mg; and anti-oxidant (ethoxyquin), 100 mg.

[2] Calculated from the data provided by the Dutch Bureau of Livestock Feedingstuffs (1994)

The diets were fed *ad libitum*. Water was also available *ad libitum* via an automatic device. After termination of the performance trial, four randomly selected birds per cage (= 24 birds per treatment group) were killed by injection of T 61 (Embutramide-Mebezoniumiodide-Tetracainhydrochloride mix, Hoechst, G). Food and water remained available to these birds until sacrifice. After sacrifice, the small intestine was removed immediately and samples three cm in length were taken from the intestinal wall, 15 cm distal of the Meckels diverticulum, for measuring morphological characteristics. These measurements were carried out in pooled samples of two birds each. In addition, samples of the digesta of the ileum were taken for measuring the viscosity, pH and bacterial counts. These samples were taken from the Meckels diverticulum to the end of the small intestine. Analyses in the ileal digesta were performed in pooled samples of four chicks (taken one from each cage).

Analytical methods

Morphological characteristics. Each sample of the small intestine was cut open longitudinally at the antimesenteric attachment. Samples were fixed on dental wax with the villi on the upper side and fixed in 0.1 M-phosphate-buffered formalin solution (40 ml/l). The shape of the villi was studied with a dissecting microscope and characterized according to a previously described classification (van Leeuwen *et al.*, in press). The following classes of villi were distinguished: tongue-shaped villi, finger-shaped villi, leaf-shaped villi, ridge-shaped villi and convoluted villi.

After the microscopic study, a 3 mm wide zone from the mesenteric site was cut at right angles to the surface of the mucosa and embedded in paraffin wax. Sections were cut (5 µm) and stained with haematoxylin and eosin (HE staining), a combination of the periodic acid-Schiff method (PAS staining) with the basophilic dyes alcian blue (AB staining), and a combination of the basophilic dyes high iron diamine (HID staining) and the basophilic dyes alcian blue (AB staining). From these stained sections, villus height, crypt depth, villi:crypt ratio, index of mitosis (meta- and anaphases) per 100 crypt cells were determined according to the procedure of Kik *et al.* (1990). In addition, also the goblet cells, classified as neutral or acidic cells and sialo- or sulpho-cells, respectively were determined per 100 µm crypt and number per crypt.

Ileal viscosity. The viscous property of the intestinal chyme was determined with a Brookfield digital viscometry (Model LVTD VCP-II, Brookfield Engineering Laboratories, Inc., USA), according to the method of Bedford and Classen (1993).

pH. The pH of the aqueous fraction was measured in the same sample in which the ileal viscosity was determined. The pH was measured by inserting a micro pH-electrode (LoT 440-M3, Dr. W. Ingold AG, Udorf, Germany) into the aqueous fraction. Measuring of the pH was done immediately after collection of the samples in order to minimize carbonate buffering.

Bacterial counts. Immediately after the samples were pooled, 2 g of fresh chyme were collected for microbial measurements and put into pre-weighed bottles with 18 ml anaerobic transport medium (TRM) containing 850 ml distilled water, 150 ml glycerol (Merck), 5 g/l yeast extract (Oxoid), 1 g/l peptone (Oxoid), 8.5 g/l sodium-chloride (Merck), and 0.5 g/l L-cystine-Cl (BDH). The pH of TRM was 7.0 ± 0.1. The samples were weighed and stored at 4 ± 1 °C. In an anaerobic glove box the samples were homogenized, pipetted into 4 marked cryotubes (2 ml), and stored in liquid nitrogen. After thawing at 37°C in the anaerobic glove box, 10-fold dilutions were made in Peptone Physiological Saline. Aliquots of 0.1 ml were spread onto the following agar media: Reinforced Clostridial Agar (RCA, Oxoid CM151) supplemented with 5 g/l glucose, and, after sterilization, 75 ml/l sterile horse blood and 75 ml (0.4%)/l China blue (RCB) agar for total anaerobic bacteria; RCA agar containing 80 ml/l kanamycin and 1 ml/l vancomycin, and, after sterilization, 75 ml/l sterile horse blood for *Bacteroidaceae*; Eugon agar (BBL 11230) supplemented with 10 g/l maltose (Merck), 400 ml vegetable (tomato) juice (Campbell V8) and, after sterilization, 5 ml/l sterile propionic acid to bring the pH at 6.0 ± 0.2 for *Bifidobacterium*. These culture media were incubated anaerobically in gas tight plastic bags (Merck) at 37°C for 120 h.

Outside the anaerobic glove box aliquots of 92 µl were spread by spiral plating (Spiral System Instruments, Bethesda, USA) onto the following agar media: Trypton Soy Broth (Oxoid CM 129) supplemented with 15 g/l agar (Oxoid) for total aerobic bacteria; Rogosa agar (Oxoid CM627) for *Lactobacillus*; Perfringens agar base (Oxoid CM587) with 2 vials/l Perfringens SFP selective supplement (Oxoid SR93) and 50 ml/l egg yolk emulsion (Oxoid SR47) for *Clostridium*; Slanetz and Bartley medium (Oxoid CM377) for *Enterococcus*; Violet Red Bile Glucose agar (Oxoid CM485) for *Enterobacteriaceae*; These culture media were incubated anaerobically (GasPak) or aerobically at 37°C for 24 to 72 h. After incubation the specific colonies on the selective culture media were counted and the number of viable colony forming units per g chyme sample (CFU/g) were calculated. Based on the morphology of the microorganisms on the agar media for *Enterobacteriaceae*, the *Escherichia coli* were calculated.

Experiment 2: Digestibility trial

This experiment was conducted to estimate the digestibility of nutrients and the metabolizable energy of the three diets used in the performance trial. The birds for this study originated from those used in the performance trial. After the completion determination of the performance trial (at 21 d of age), 32 birds from each of the three preceding treatments were selected to maintain the differences in body weights among the treatments. The same diets as used in the performance trial were fed to these birds (Table 1). In all diets a marker (15 g Diamol/kg) was included for measuring digestibility. Each treatment included 4 cages of 8 birds each.

The birds were housed under conditions similar to those described in the growth trial. The digestibility study included a pretest period of 5 d (age period 21 to 26 d) and a test period of 4 d (age period 26 to 29 d). During the pretest period birds were accustomed to water troughs, in order to determine water consumption. During the test period, excreta were collected quantitatively on plastic trays in each cage during the 4 x 24 h time period. Contaminations, such as down and scales, were carefully removed and the excreta were collected and stored in closed containers at -20 °C. During the day (08.00-20.00 h), excreta were collected at intervals of 4 h, and one during the night (20.00-08.00 h) at 08.00 h. Day and night excreta were collected separately. Only the excreta collected during the day were used for the analyses. Previous studies (van der Klis *et al.*, 1995, unpublished) indicated that there were no significant differences in digestibility when excreta were collected over a 12 h or a 24 h period per day.

Birds were deprived from food during 6 h before the start of the test period and 6 h before the end of the test period. Food was available ad libitum to the birds during the remaining time. Food intake during the test period was determined daily for each cage of the 8 birds. Water was also available ad libitum; water consumption was measured for each cage separately at intervals of 12 h. Water intake was measured as the difference of a predetermined volume of water in the trough and that remaining in the trough.

The three experimental diets and the excreta that was collected daily were analysed for dry matter, nitrogen, fat, starch, ash and gross energy (GE). In addition, the dry matter content of the excreta that was produced at night was determined and used for calculating total dry matter output per 24 h. The AME of each diet was calculated from the GE values of the diet and excreta. The AME values were corrected to zero nitrogen balance (AME_n). The correction factor used was 8.22 kcal/g of retained nitrogen as proposed by Hill and Anderson (1958).

Chemical analysis

Samples of feed and freeze-dried excreta were milled to pass through a 1.0 mm screen before analysis. All analyses were carried out in duplicate. Dry matter content was determined by drying the samples to a constant weight at 101°C. Inorganic matter and N were determined by standard methods (Association of Official Analytical Chemists, 1984), GE was determined by using an IKA-C4000 adiabatic colorimeter. Crude fat was determined by treating for 1 h with 4 M hydrochloric acid and drying for 3 h under vacuum at 100°C, followed by 8 h extraction with hexane. Starch content was determined enzymatically by hydrolysing of the starch to glucose with amyloglucosidase (NEN 3574). Amino acids were determined by ion-exchange chromatography (Slump, 1969) after hydrolysis of the samples with 6 M hydrochloride for 24 h at 100 °C. Methionine and cystine were determined by ion-exchange chromatography as methionine sulphate and cysteic acid, respectively, after oxidation with performic acid. Tryptophan was not determined.

Statistical analysis

Data were subjected to analysis of variance using the SPSS/PC + V5.0 computer program of Norusis (1992). Treatment factors were type of diet and battery tier. The treatment means were tested for difference by use of the Least Significant Difference test (Snedecor and Cochran, 1980). All statements of significance are based on a probability of less than 0.05.

Results

Experiment 1, Growth performance trial

Mortality rate was low as only 1.7% of the birds died with no appreciable differences in mortality being observed among the treatment groups. The addition of HMC to the diet depressed weight gain (19%; $P < 0.05$) and food utilization (37%; $P < 0.05$; Table 2). Inclusion of LMC in the diet had only a small negative effect on weight gain (2%) and food utilization (3%), which were of no significance importance. The addition of both HMC or LMC to the diet significantly increased water intake. As a consequence, water:food ratio was also increased significantly when either of the two pectin products were included in the diet.

The digesta of birds fed the HMC diet was gel like and had a high waterbinding capacity. Digesta viscosity and pH could not be measured as no supernatant was obtained after centrifugation (10,000 x g for 10 min at 4 °C) of the digesta, but was certainly very high.

Table 2. *Effect of high-methylated citrus pectin (HMC) and low-methylated citrus pectin (LMC) on chick growth performance from 1 to 21 d of age (Experiment 1) and on water consumption[1] (Experiment 2).*

Diet	Weight gain (g)	Food intake (g/bird/d)	Food/gain ratio (g:g)	Water intake (g/bird/d)	Water/food ratio (g:g)
Corn	766^a	47.4^a	1.301^b	226^c	1.83^c
Corn + 30 g HMC/kg	618^b	52.4^b	1.782^a	331^a	2.43^a
Corn + 30 g LMC/kg	750^a	47.8^a	1.338^b	290^b	2.32^b
SEM (df=13)*	16.3	1.19	0.0156	10.4	0.032

[a,b] Mean values within a column with no common superscript differ significantly ($P \leq .05$).

* Except for water intake and water/food ratio (df = 8).

[1] Means of 4 d test period (age period 21 - 26 d).

The inclusion of HMC in the diet increased significantly the total number of aerobic counts as well as the numbers of *Enterococci*, *Bacteroidaceae*, *Clostridium* and *E. coli* (Table 3). Inclusion of LMC in the diet increased digesta viscosity and ileal pH significantly. Inclusion of LMC in the diet had a minor effect on microbial activity. Only the number of *Clostridium* in the ileal chyme was increased ($P < 0.05$).

Addition of HMC to the diet affected the shape of the villi in the ileum (Table 4). The number of zigzag patterns and ridge-shaped villi were significantly reduced in birds fed the HMC diet compared to these present in birds fed the control diet, whereas the number of tongue-shaped villi was significantly increased ($P < 0.05$). No significant change was found in villus length when HMC was included in the diet. The numbers of goblet cells in the villi, however, were 66 and 48% higher, respectively, in the HMC and LMC treated birds compared to these present in birds fed the control diet. The HMC diet was also associated with a higher sucrase-isomaltase activity ($P < 0.05$) than that obtained in birds fed the control diet.

Experiment 2, Digestibility trial.

The addition of HMC to the diet significantly reduced ($P < 0.05$) digestibility of dry matter, organic matter, crude fat, starch, N-retention and the metabolizable energy value of the diet (Table 5). Inclusion of LMC in the diet significantly reduced ($P < 0.05$) fat and ash digestibility and metabolizable energy value of the diet. Dry matter content of the excreta was reduced significantly ($P < 0.05$) when HMC or LMC was included in the diet. Digestibility of amino acids was significantly ($P < 0.05$) depressed when HMC was added to the diet (- 16.4%), whereas inclusion of LMC in the diet only tended to reduce the digestibility of amino acids (- 1.9%; Table 6). The reductions in digestibility of the individual amino acids varied between 22.9 and 8.4% in the HMC diet and between 7.1 and 1.0% in the LMC diet.

Table 3. *Effect of high-methylated citrus pectin (HMC) and low-methylated citrus pectin (LMC) on digesta viscosity, ileal pH and colony forming units of anaerobic and aerobic bacterial species (log cfu/g) per g intestinal chyme of 21 d old broiler chicks (experiment 1).*

	Dietary treatments			SEM
	corn	corn + 30 g HMC/kg	corn + 30 g LMC/kg	(df=13)*
Digesta viscosity (cP[1])	3.1^{b}	nm	6.0^{a}	0.39
pH	6.8^{a}	nm	6.4^{b}	0.11
Total aerobic colony counts	6.8^{b}	7.4^{a}	6.6^{b}	0.19
Total anaerobic colony counts	7.2	7.7	7.3	0.35
Bifidobacterium spp	6.9	7.4	7.4	0.24
Lactobacillus spp	7.1	6.4	6.8	0.29
Enterococcen spp	4.9^{b}	6.1^{a}	4.5^{b}	0.40
Bacteroidaceae	4.2^{b}	5.5^{a}	3.6^{b}	0.36
Clostridium spp	1.5^{c}	3.8^{a}	2.6^{b}	0.36
E. coli	5.4^{b}	7.0^{a}	4.9^{b}	0.24

nm; not measurable

^{a,b} Mean values within a row with no common superscripts differ significantly ($P \leq .05$).

* Except for viscosity and pH (df = 8).

[1] cP = centiPoise (1 cP = 1/100 dyne sec/cm^2 = 1 mPa.s).

Table 4. *Effect of high-methylated citrus pectin (HMC) and low-methylated citrus pectin (LMC) on morphological classification of the villi, histological measurements and histochemical criteria in the ileum of 21 d old broiler chicks (experiment 1).*

Treatment	Corn	Corn + 30 g HMC/kg	Corn + 30 g LMC/kg	SEM (df=13)
Classification of the villi (% of total)				
Zigzag	62^{a}	5^{b}	50^{a}	8.9
Shape of the villi (% of total)				
Tongue-shaped	15^{b}	43^{a}	12^{b}	4.8
Leaf-shaped	43	47	45	5.8
Ridge-shaped	40^{a}	2^{b}	36^{a}	6.4
Convoluted-shaped	3	9	7	3.8
Histological measurements				
Villus length, µm	844	771	870	38.8
Crypt depth, µm	133	129	131	7.6
Villus/crypt ratio, abs.	6.5	6.1	6.8	0.428
Number of goblet cells per 100 villus cells, %	29^{b}	48^{a}	43^{a}	4.27
Number of goblet cells per 100 crypt cells, %	53	51	53	6.45
Histochemical criteria				
Acid/base gradation of mucin, abs.	1.67	1.42	1.92	0.419
Sulpho mucin in villus, %	100.0	95.4	100.0	1.7
Sialo mucin in villus, %	0.0	4.6	0.0	1.7
Sucrase-isomaltase activity, U/g	74^{b}	100^{a}	90^{ab}	5.7

[a,b] Mean values within a row with no common superscripts differ significantly ($P \leq .05$).

Discussion

The present study showed that addition of HMC to a corn-based diet depressed broiler performance, whereas inclusion of LMC in the diet had only a small negative effect on broiler performance. These results are in agreement with the findings of a previous study (Langhout and Schutte, 1996). Results of the present study indicate that the observed reduction in performance in the HMC fed birds was at least partly the result of a decrease in nutrient digestibility, particularly that of fat. As a consequence the metabolizable energy content of the diet was reduced. The small negative effect of LMC on performance seems to be mainly the result of a reduced fat digestibility as found in the present study. However, it should be noted that the results of the performance trial were not completely comparable with those of the digestibility trial. The performance data were obtained from birds 1 - 21 days of age,

Table 5. *Effect of high-methylated citrus pectin (HMC) and low-methylated citrus pectin (LMC) on the digestibility, metabolizable energy content, N retention, and dry matter content of the excreta (experiment 2).*

	Corn	Corn + 30 g HMC/kg	Corn + 30 g LMC/kg	SEM (df=8)
Digestibility coefficients				
- dry matter	65.9^a	55.3^b	63.2^a	1.10
- organic matter	68.0^a	56.8^b	65.5^a	1.35
- crude fat	87.9^a	46.2^c	78.5^b	1.61
- starch	96.2^a	87.3^b	95.8^a	0.99
- ash	34.2^a	34.0^a	27.0^b	1.17
N retention (% of intake)	50.7^a	43.2^b	49.0^a	1.27
Metabolizable energy (ME$_n$, MJ/kg)	12.91^a	10.13^c	12.25^b	0.192
Dry matter content of excreta	28.3^a	25.9^b	22.6^c	0.0065

[a,b,c] Mean values within a row with no common superscripts differ significantly ($P \leq .05$).

whereas the digestibility data were determined in four week old chicks. It is well known that the capacity of young birds to digest nutrients increases with age (Nir *et al.*, 1993; Nitsan *et al.*, 1994). In particular this is true for the digestibility of fat (Ketels and de Groote, 1988). In addition, the anti-nutritive effects of water-soluble NSP are more pronounced in young birds than in older birds (Marquardt *et al.*, 1979; Veldman and Vahl, 1994).

It has been reported previously that HMC caused sticky droppings (Wagner and Thomas, 1977; Patel *et al.*, 1980). In the present study both HMC and LMC increased water consumption, and reduced dry matter content in the excreta. The latter was more pronounced when LMC was added to the diet, whereas the water/food ratio was similar for both pectin products. This can be explained by the fact that in the HMC fed birds both water and dry matter excretion were increased.

The mechanism by which viscous NSP such as HMC and to a lesser extent LMC exhibit their anti-nutritive properties is not well understood. It is suggested that due to an increase in digesta viscosity, more undigested nutrients enter the lower part of the small intestine. As a result microbial growth will be increased and the competition between the host and the bacteria for nutrients will favour the bacteria. This is more or less supported by the results of Choct *et al.* (1996), who reported that the volatile fatty acid concentration in the ileum was increased by including wheat pentosans in chick diets. This increase in volatile fatty acid concentration suggests an increased microbial activity. In the present study HMC markedly affected microbial composition in the small intestine, particularly that of *Enterococci*, *Bacteriodaceae*, *Clostridia* and *E. coli*, while LMC only increased the number of *Clostridia*. This difference in effect on microbial activity between LMC and HMC may be associated with the lower anti-nutritive properties of LMC as compared to HMC.

Beside the competition for nutrients, the microflora may also inhibit fat digestibility. Some species of bacteria can deconjugate bile acids (Hylemond, 1984). During deconjugation the acid-amide binding is hydrolysed by either cholylglycine or cholyltaurine hydrolase. To support this theory, Feighner and Dashkevicz (1988) showed that a rye-based diet had a higher cholyltaurine level than a maize-based diet, suggesting that the deconjugation rate was increased in the rye-based diet. Bile acids are an essential component of micelle formation and hence fat digestion. As a result, an increase in the activity of these bacteria significantly reduces fat digestibility. This effect is even more pronounced for long chain and saturated fatty acids (Ward and Marquardt, 1983), since short chain or unsaturated fatty acids are more easily absorbed without the forming of micelles (Garrett and Young, 1975).

Table 6. *Effect of high-methylated citrus pectin (HMC) and low-methylated citrus pectin (LMC) on amino acid digestibility (experiment 2).*

	Corn	Corn + 30 g HMC/kg	Corn + 30 g LMC/kg	SEM (df=8)
Isoleucine	82.8^a	66.7^b	81.8^a	0.64
Leucine	86.1^a	65.0^b	85.1^a	0.75
Lysine	84.6^a	76.1^c	83.0^b	0.47
Methionine	89.4^a	77.0^b	88.1^a	0.49
Cystine	68.2^a	46.6^c	61.1^b	0.71
Methionine + cystine	81.1^a	65.2^c	77.8^b	0.54
Phenylalanine	77.2^a	57.4^c	74.3^b	0.73
Tyrosine	83.2^a	65.3^c	80.9^b	0.59
Threonine	75.9^a	57.2^c	72.6^b	0.70
Valine	81.3^a	63.7^b	79.3^a	0.69
Arginine	86.9^a	76.2^b	85.9^a	0.36
Histidine	81.7^a	68.2^c	79.1^b	0.71
Alanine	82.8^a	63.1^b	80.6^a	0.72
Aspartic acid	78.4^a	63.8^c	76.1^b	0.47
Glutamic acid	86.8^a	72.2^b	85.7^a	0.53
Glycine	73.0^a	55.7^c	69.2^b	0.66
Proline	82.5^a	59.7^c	79.7^b	0.62
Serine	80.3^a	63.5^c	77.8^b	0.67
Mean amino acids	82.6^a	66.2^c	80.7^b	0.56

^{a,b,c} Mean values within a row with no common superscripts differ significantly ($P \leq .05$).

Another hypothesis is that the increase in microbial activity resulting from water-soluble NSP may influence gut morphology and consequently affect nutrient absorption. In the present study villi of birds fed the control diet were mainly ridge-shaped and leaf-shaped and arranged in a zigzag pattern. This is in accordance with the findings of Lim and Low (1977), who showed that in young birds villi appear as zigzag ridges. Inclusion of HMC in the diet markedly affected the shape and the arrangement of the villi, and tended to reduce the length of the villi, which indicates that the absorptive area was reduced. It has been suggested that a shorter villi relative to crypt depth have fewer absorptive cells but and more secretory cells

(Schneeman, 1982). The latter conclusion corresponds with the findings of the present study as the inclusion of HMC or LMC in the diet increased in the number of goblet cells per 100 villus cells. Goblet cells are responsible for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium (Schneeman, 1982). Thus, a higher density in goblet cells may result in an increase in the secretion of mucin. Changes in mucin content or the composition of the mucosal surface may also decrease nutrient absorption or increase energy requirement for gut maintenance. An increase in cell turnover is associated with a reduction in the maturity of the goblet cells. A reduction in maturity of these cells resulted in an increase in the number of sialo-mucin containing goblet cells and a decrease in the number of sulpho-mucin containing goblet cells (Filipe, 1977; Culling *et al.*, 1981). In the present study, however, hardly any differences in sialo-mucin and sulpho-mucin containing goblet cells were observed between the groups, whereas the sucrase-isomaltase activity was increased by including HMC or LMC in the diet. Kik *et al.* (1990) associated morphological abnormalities in pigs with a reduction in the activity of brush border enzymes. This could not be confirmed in our study. However, differences in effects between literature data and our data may be related to differences between animal species.

Based on the results of the present study, it can be stated that water-soluble pectins in diets of young chicks changes microbial activity of the ileum and morphology of the small intestinal wall. The magnitude of these changes depends on the degree of methylation of the pectins.

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References

Antoniou, T., Marquardt, R.R. & Cansfield, P.S. (1981) Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agricultural Food and Chemistry* 28: 1240-1247.

Association of Official Analytical Chemists (1984) *Official Methods of Analsis*, 14th edn (Washinton, DC, AOAC).

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens; effect of microbial status, diet gamma irradation and sodium taurocholate supplementation *British Poultry Science* 24: 191-203.

Choct, M. & Annison, G. (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Moran, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Classen, H.L., Campbell & GroodWassink, W.B. (1988) Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. *Canadian Journal of Animal Science* 68: 1253-1259.

Culling, C.F.A., Reid, P.E., Dunn, W.L. & Freeman, H.J. (1981) The relevance of the histochemistry of colonic mucins based upon their PAS reactivity. *Histochemical Journal* 13: 889-903.

Dutch Bureau of Livestock Feedingstuffs (1994) Chemical composition, digestibility and energy value of feed ingredients. Bureau of Livestock Feedingstuffs, Lelystad, The Netherlands.

Feighner, S.D. & Dashkevicz, M.P. (1988) Effect of dietary carbohydrates on bacterial cholyltauryl hydrolase in poultry intestinal homogenates. *Applied Environmental Microbiology* 54: 337-342.

Filipe, M.L. (1977) Mucin histochemistry in the detection of early malignancy in the colonic epithelium. *Advances in Experimental Medicine and Biology* 89: 485-489.

Fincher, G.B. & Stone, B.A. (1986) Cell walls and their components in cereal grain technology. In: *Advances in Cereal Science and Technology, Vol VIII* (Ed. Pomeranz, Y.), Minnesota, AACC, pp. 207-295.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Gee, J.M., Lee-Finglas, W., Wortley, G.W. & Johnson, J.T. (1996). Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. *Journal of Nutrition* 126: 373-379.

Hesselman, K. & Åman, P. (1986) The effect of ß-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. *Animal Feed Science and Technology* 15: 83-93.

Hill, F.W. & Anderson, D.L. (1958) Comparison of metabolizable energy and productive energy determinations with growing chicks *Journal of Nutrition* 64: 587-603.

Hofshagen, M. & Kaldhusdal, M. (1992) Barley inclusion and avoparcin supplementation in broiler diets. 1. Effect on small intestinal bacterial flora and performance. *Poultry Science* 71: 959-969.

Hylemond, P.B. (1985) Metabolism of bile acids in intestinal microflora In: Sterols and bile acids: New Comprehensive Biochemistry (Danielsen, H. & Sjövall, J., eds.) Elsevier Science Publishers B.V. pp 331-343.

Ketels, E. & De Groote, G. (1988) The nutritional value for broilers of fats characterized by short-chain fatty acids as affected by level of inclusion and age. *Animal Feed Science and Technology* 22: 105-118.

Kik, M.J.L., Huisman, J., Van der Poel, A.F.B. & Mouwen, J.M.V.M. (1990) Pathologic changes of the small intestinal mucosa of pigs after feeding Phaseolus vulgaris beans. *Veterinary Pathology* 27: 329-334.

Langhout, D.J. & Schutte, J.B. (1996) Nutritional implications of pectins in chicks in relation to esterification and origin of pectins. *Poultry Science* 75: 1236-1242.

Lim, S. & Low, F.N. (1977) Scanning electron microscopy of the developing canal of the chick. *American Journal of Anatomy* 150: 149-154

Marquardt, R.R., Ward, A.T. & Misir, R. (1979) The retention of nutrients by chicks fed rye diets supplemented with amino acids and penicillin. *Poultry Science* 58: 631-640.

National Research Council (1994) Nutrient requirements of poultry, 9th revised edn (Washington DC, National Academy of Sciences).

Nir, J., Nitsan, Z. & Mahagan, M. (1993) Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. *British Poultry Science* 34: 523-532.

Nitsan, Z., Ben-Aviaham, G., Zoref, Z. & Nir, J. (1991) Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. *British Poultry Science* 32: 515-523.

Norusis, M.J. (1992) SPSS/PC+ Base System User's Guide Version 5.0 (SPSS inc., Chicago, Illinois).

Patel, M.B., Jami, M.S. & McGinnis, J. (1980) Effect of gamma irradiation, penicillin and or pectic enzyme on chick growth depression and fecal stickiness by rye, citrus pectin and guar gum. *Poultry Science* 59: 2105-2110.

Sakata, T. (1987) Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *British Journal of Nutrition* 58: 95-103.

Schneeman, B.D. (1982) Pancreatic and digestive function. In: Dietary Fibre in Health and Disease (Vahouny, G.V. & Kritchevsky, D. eds.) Plenum Press, New York, pp. 73-83.

Silva, S.S. & Smithard, R.R. (1996) Exogenous enzymes in broiler diets: crypt cell proliferation, digesta viscosity, short chain fatty acids and xylanase in the jejunum. *British Poultry Science* 37 (supplement): S77-S79.

Slump, P. (1969) Karakterisering van de voederwaarde van eiwitten in voedermiddelen door hun aminozuursamenstelling en de invloed van verhitting en loogbehandeling op de benutbaarheid van aminozuren. *PhD Thesis*, Free University Amsterdam, Amsterdam, The Netherlands.

Smits, C.H.M. (1996) Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. PhD thesis, Agricultural University Wageningen, the Netherlands.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical methods. 7th ed. (The Iowa State University Press. Ames, 1A).

Veldman, A. & Vahl, H.A. (1994). Xylanase in broiler diets with differences in characteristics and content of wheat. *British Poultry Science* 35: 537-550.

Viveros, A. Brenes, A., Pizarro, M. & Castano, M. (1994) Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Animal Feed Science and Technology* 48: 237-251.

Wagner, D.D. & Thomas, O.P. (1977) A rye type growth depression of chicks fed pectin. *Poultry Science* 56: 615-619.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

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

Ward A.T. & Marquardt, R.R. (1987) Antinutritional activity of a water-soluble pentosan-rich fraction from rye grain. *Poultry Science* 66: 1655-1674.

Chapter 5

Effect of dietary viscous polysaccharides on the ileal microflora and on bile acid deconjugation in broiler chicks

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Abstract

A study was conducted with broiler chicks to investigate the effects of high methylated citrus pectin (HMC), low-methylated citrus pectin (LMC), wheat and rye on activity and composition of the microflora and degree of bile acid deconjugation in the small intestine in relation to the fat source. Two basal diets were used, a diet containing 450 g corn/kg and a diet containing 250 g rye/kg and 250 g wheat/kg. Both diets were either supplemented with 65 g soya oil/kg or 5 g soya oil/kg and 60 g blended animal fat/kg. Both pectin sources were incorporated into the corn-based diet at a concentration of 0 or 30 g/kg. The inclusion of HMC, LMC or rye/wheat in the diet significantly depressed weight gain and food utilization in chicks. The depression in performance was more pronounced when the diet contained blended animal fat instead of soya oil. The addition of HMC, LMC or rye/wheat to the diet reduced significantly the pH of the ileal chyme, whereas the concentration of volatile fatty acids in the ileal digesta was increased only in birds fed the rye/wheat diet ($P < 0.05$). The inclusion of HMC in the diet increased significantly the total count of aerobic and anaerobic microflora including the numbers of *Bifidobacteria*, *Enterococci* and *Escherichia coli*. When birds were fed the rye/wheat-based diet only the number of *E.coli* were raised ($P < 0.05$). Inclusion of LMC in the diet did not greatly affect the microbial population or composition. The concentration of conjugated bile acids was significantly reduced when HMC, LMC or rye/wheat were included in the diet, whereas the corresponding concentration of unconjugated bile acids was increased ($P < 0.05$). The results suggest that in addition to digesta viscosity an increase in microbial activity in the small intestine is at least partly associated with the anti-nutritive properties of water-soluble non-starch polysaccharides.

Introduction

There is ample evidence that some non-starch polysaccharides (NSP) such as pentosans in wheat, and rye have anti-nutritive properties in chicks. Some of these pentosans and pectins are soluble in aqueous media and form highly viscous solutions. The water-soluble pentosans are assumed to be primarily responsible for the anti-nutritive activity in chicks, producing a depression in performance and digestibility of nutrients (Fincher and Stone, 1986; Bedford and Classen, 1993; Choct and Annison, 1990, Smits, 1996). In particular, fat digestion is negatively affected by viscous water-soluble NSP (Antoniou *et al.*, 1981; Campbell *et al.*, 1983; Choct and Annison, 1992).

It is well known that dietary ingredients may modify these anti-nutritional effects of pentosans and pectins in chicks (Wagner and Thomas, 1978; Marquardt *et al.*, 1979; Antoniou *et al.*, 1980). Antoniou *et al.* (1980) reported that performance and fat digestibility were considerably depressed in rye-fed birds when the fat type was tallow, but these effects were only small when soya oil was used. From the results of a subsequent study Antoniou and Marquardt (1982) concluded that the utilization of dietary fat by rye-fed birds depends on the degree of saturation and chain length of fatty acids, concentration of dietary lipids, and the microbial activity in the small intestine. With regard to the latter, Wagner and Thomas (1978) reported that there was a marked increase in microbial activity in the small intestine of chicks when rye or citrus pectin was included in the diet. Langhout *et al.* (1998) reported also an increase in microbial activity in the small intestine of chicks when high methylated citrus pectin was included in the diet. An increase in ileal microbial activity may increase degradation of conjugated bile acids. Thereby making fat emulsification less effective (Coates *et al.*, 1981). Long chain saturated fatty acids are mostly absorbed in the ester form and not as free fatty acids (Garrett and Young, 1975). Therefore, a reduction in fat absorption would be more pronounced in chicks fed diets containing saturated fats such as tallow than in diets containing unsaturated fats such as soya oil.

The objectives of present study was to obtain additional information about the effects of certain water-soluble NSP and type of fat on the microbial activity and the degree of deconjugation of bile acids in the small intestine of chicks. The water-soluble NSP containing products involved in this study were high- (HMC) and low-methylated citrus pectin (LMC) as model carbohydrates, and wheat and rye as a source of high viscous water-soluble pentosans. The fat sources tested were blended animal fat as a source of a saturated fat and soya oil as a source of unsaturated fat.

Materials and methods

Experimental design

Two basal diets were used, one based on corn and the other on rye and wheat (Table 1). All basal diets were formulated to meet the requirements of broiler chicks (National Research Council, 1994). Additives such as antibiotics and coccidiostats were omitted from the diets, as they may interfere with microbial activity. Thirty grams of highly methylated citrus pectin (HMC; type CU 301, methylation > 65%, Contined b.v., The Netherlands) or low-methylated citrus pectin/kg (LMC; type CU 12, methoxylation 30 to 38%, Contined b.v., The Netherlands) were added to the corn-based diet. The pectin products, which were supplied as anhydrous polysaccharides were substituted in the diets by weight for wood cellulose. The ME value of wood cellulose and pectin was assumed to be zero. All basal diets were either supplemented with 65 g soya oil/kg (containing 85% unsaturated fatty acids) or 5 g soya oil/kg and 60 g blended animal fat/kg (containing 55% unsaturated fatty acids). The difference in calculated ME values between the soya oil diets and the blended animal fat diets was corrected with tapioca and wood cellulose.

One-day-old male broiler chicks ("Ross") were housed in three tiered, electrically heated battery cages having wire floors each with a floor space of 975 cm^2. The cages were located in an insulated room with controlled temperature and humidity. Chicks were subjected to continuous fluorescent illumination. Each treatment group was allotted *at random* to 6 cages each with 24 birds. The experimental diets were fed for a period of 21 d (1 to 21 d of age). Chicks were weighed individually and food consumption for each cage was recorded at the end of the trial. During the last 4 days of the experimental period (age period 17 - 21 d), water consumption was measured separately for each cage at intervals of 12 h. Water intake was the difference between a predetermined volume of water in the trough and that remaining in the trough. In order to determine the water/food ratio, food intake was also recorded when water intake was measured. Food (as pellets) and water were available *ad libitum*.

Table 1. *Composition of the basal diets in g/kg.*

Ingredient	Corn-based	Rye/wheat-based
Corn	450	-
Wheat	-	250
Rye	-	250
Tapioca	43.1	40
Soya oil	65	65
Animal fat	-	-
Soyaflour (50%)	230	220
Corngluten meal	50	50
Fishmeal (70.6%)	10	10
Meat meal tankage (58%)	40	40
Cellulose (Arbocel B 800)	65	30
Molasses	10	10
Limestone	9.2	9.4
Monocalcium phosphate	11.2	10
Salt	2.5	2.4
Vitamin + minerals[1]	10	10
L-lysine	2.2	1.7
DL-methionine	1.8	1.5
Calculated contents[2]:		
Crude protein	221	228
Metabolizable energy (MJ/kg)	12.22	11.72
Ca	8.6	8.6
P	6.5	6.5
Lysine	12.4	12.4
Methionine + cystine	9.0	9.2

[1] Supplied per kg diet: riboflavin, 4 mg; niacinamide, 40 mg; d-pantothenic acid, 12 mg; choline-chloride, 500 mg; cobalamin, 15 μg; Dl-α-tocopheryl acetate, 15 mg; menadione, 5 mg; retinyl- acetate, 3.44 mg; cholecalciferol, 50 μg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.7H_2O$, 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 100 mg; $ZnSO_4.H_2O$, 150 mg; Na_2SeO_3, 0.15 mg; KI, 5 mg; $CoSO_4.7H_2O$, 1 mg; antioxidant (ethoxyquin), 100 mg

[2] Calculated from the data provided by the Dutch Bureau of Livestock Feedingstuffs (1995).

Six randomly selected birds per cage (= 36 birds per treatment group) were also used for additional studies after termination of the trial. Food and water remained available to these birds until sacrifice at 22 d of age, after which they were weighed individually. After sacrifice, by injection of T 61 (Embutramide-Mebezoniumiodide-

Tetracainhydrochloride mix, Hoechst, G), the small intestine was removed immediately and the weight of the duodenum plus jejunum and ileum was determined. In addition, caecal weight including their contents was determined. Samples of the ileal contents (digesta) were taken from the Meckels diverticulum to the end of the small intestine for determination of viscosity, pH, content of volatile fatty acids (VFA), bacterial counts and content of bile acids. Analyses of the digesta were performed in pooled samples of six chicks each taken from a different cage.

Analytical methods

Ileal viscosity. The viscous property of the intestinal chyme was determined with a Brookfield digital viscometry (Model LVTD VCP-II, Brookfield Engineering Laboratories, Inc., USA), according to the method of Bedford and Classen (1993).

pH. The pH was measured by inserting a micro pH-electrode (LoT 440-M3, Dr. W. Ingold AG, Udorf, Germany) into the ileal chyme.

Volatile fatty acids. Concentration of VFA (acetic, propionic, iso-butyric, butyric, iso-valeric and valeric) in ileal digesta was determined according to the gas-liquid chromatographic method (GLC; Model HP 5890, Hewlett Co. Palo Alto, CA 94304) of Langhout and Schutte (1996).

Bacterial counts. Immediately after the samples were pooled, 2 g of fresh chyme were collected for microbial measurements according to the method of Langhout *et al.* (1998).

Bile acids. A sample of 250 mg of ileal digesta was mixed with two internal standards, 7α,12α-dihydroxy-5β-cholanoic acid (0.5 μmol) for the unconjugated bile acids and tauro-7α,12α-dihydroxy-5β-cholanoic acid (0.5 μmol) for the conjugated bile acids. The sample was saponified according to Faassen *et al.* (1985). After addition of 1 mL of 10 M NaOH and 6 mL of methanol, the mixture was refluxed for 2 h. After refluxing the mixture was centrifuged and the supematant was neutralized with 1 mL of concentrated HCL and 18 mL of phosphate buffer (0.5 M, pH 7.0). Next, the bile salts were extracted into their unconjugated and conjugated fractions using Lipidex-DEAP chromatography as described by Tangerman *et al.* (1986). After enzymatic hydrolysis, methylation and solylation, the concentration of conjugated and unconjugated bile acids was determined according to the gas chromatography as described by Salemans *et al.* (1993). Prior to the measurements of bile acids samples of ileal digesta were subjected to solvolysis (Princen *et al.*, 1990) in order to determine whether or not bile salts were sulphated. These analyses demonstrated that the samples did not contain significant amounts of sulphated bile salts.

Statistical analysis

Data were subjected to analysis of variance using the SPSS/PC + V5.0 computer program of Norusis (1992). The treatment factors were diet type, fat source and battery tier using the following model:

$$y = \mu + a . D + b . F + c . D . F + d . T + e$$

where y = response measurements; D = diet type, F = fat source, and T = tier of the battery. Treatment means were tested for difference by use of the Least Significant Difference test (Snedecor and Cochran, 1980). All statements of significance are based on a probability of $P < 0.05$.

Weights of the small intestinal tract and caeca, the pH, the viscosity, the concentrations of volatile fatty acids, the bacterial counts and the concentration of conjugated and unconjugated bile acids in the ileal digesta for chicks fed both fat sources were similar and statistically not significant. The same results were also obtained for the control group (corn-based diet). Therefore, the results obtained on both fat sources for these criteria were combined within each NSP source.

Results

Weight gain, food utilization and water consumption of broiler chicks fed the control diet (corn-based diet) were not affected by the type of fat (Table 2). Inclusion of HMC, LMC or rye/wheat in the diet depressed significantly weight gain and food utilization compared to birds fed the control diet. The largest depression in weight gain and food utilization was obtained in chicks fed the HMC diet, followed by these fed the rye/wheat diet. The magnitude of the anti-nutritive effects of HMC, LMC, and rye/wheat depended on the type of fat added to the diets. Weight gain and food utilization of birds fed the HMC diet were significantly more depressed when blended animal fat compared to soya oil was included in the diet. Although the weight gain for birds fed the LMC or the rye/wheat diets containing either fat source were similar; the reduction in food utilization was more pronounced in birds fed the blended animal fat diet than in those fed the diet containing soya oil. Inclusion of HMC, LMC or rye/wheat in the diet significantly increased water consumption and, as a result, the water/food ratio The effect of HMC on the water/food ratio was similar for both types of fat sources, while in birds fed the LMC or the rye/wheat diets it was increased to a greater degree when blended animal fat as compared to soya oil was

Table 2. *Effect of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC) and rye/wheat in relation to the fat source on chick performance from 1 to 21 d of age.*

Group	Diet	Fat source	Weight gain (g)	Food intake (g/bird/d)	Food/gain (g:g)	Water intake (g/bird/d)[1]	Water/food ratio (g:g)[1]
I	Corn	Soya oil	859^{a}	54.8^{a}	1.34^{a}	164^{d}	1.72^{e}
II	Corn	Animal fat	833^{a}	53.8^{ab}	1.36^{a}	166^{d}	1.77^{e}
III	Corn + 30 g HMC/kg	Soya oil	616^{d}	52.2^{bc}	1.78^{f}	193^{ab}	2.22^{b}
IV	Corn + 30 g HMC/kg	Animal fat	554^{e}	49.4^{d}	1.88^{g}	176^{c}	2.19^{b}
V	Corn + 30 g LMC/kg	Soya oil	774^{b}	50.9^{cd}	1.38^{b}	195^{ab}	2.24^{b}
VI	Corn + 30 g LMC/kg	Animal fat	761^{b}	51.9^{bc}	1.43^{c}	200^{a}	2.30^{a}
VII	Rye/wheat	Soya oil	726^{c}	53.4^{ab}	1.54^{d}	187^{b}	2.07^{d}
VIII	Rye/wheat	Animal fat	715^{c}	54.8^{a}	1.61^{e}	195^{ab}	2.13^{c}
SEM (df=38)			10.7	0.75	0.013	3.2	0.019

$^{a-g}$ Mean values within a column with no common superscripts differ significant ($P \leq 0.05$).

[1] Means of 4 d (age period 17 - 21 d).

the fat source. The mortality rate was low (2.1%) with no appreciable differences among treatments.

The addition of HMC, LMC or wheat/rye to the diet increased significantly the weights of the duodenum plus jejunum and the ileum compared to those in birds fed the control diet (Table 3). Caecal weights were significantly increased in birds fed the HMC and the wheat/rye-based diets compared to those in the control group. The inclusion of LMC in the diet did not effect caecal weights ($P > 0.05$). The pH of the ileal digesta was significantly reduced when HMC, LMC or rye/wheat was included in the diet compared to that obtained in birds fed the control diet (Table 3). The lowest pH value was found in the ileal digesta of birds fed the HMC diet followed by those fed the rye/wheat diet. The digesta of birds fed the HMC diet was gel like and had a high water-binding capacity. Digesta viscosity could not be measured as no supernatant was obtained after centrifugation (10,000 x g for 10 min 4°C) of the digesta, but it was certainly very high. Inclusion of LMC or rye/wheat in the diet increased digesta viscosity compared to that present in birds fed the control diet ($P < 0.05$). The highest digesta viscosity was measured in birds fed the rye/wheat-based diet. With regard to the VFA, only acetic and valeric acids were detected in the ileal chyme (Table 3). Inclusion of HMC in the diet had little effect on the concentration of VFA in the ileum ($P > 0.05$), whereas the addition of LMC to the diet reduced the concentration of VFA ($P < 0.05$). The reduction in VFA concentration was mainly caused by a reduction in the concentration of acetic acid. In contrast, the concentration of VFA in the small intestine was increased when birds were fed the rye/wheat-based diet as compared to that in birds fed the control diet. This was mainly the result of an increase in the concentration of valeric acid. In contrast the concentration of acetic acid was reduced.

The addition of HMC to the diet increased significantly the total number of aerobic and anaerobic microorganisms including *Bifidobacteria*, *Enterococci* and *E.coli* (Table 4). The number of *E.coli* only increased ($P < 0.05$) when birds were fed the rye/wheat containing diet. Inclusion of LMC in the diet had little effect on bacterial composition ($P > 0.05$) in this group. Only the number of *Enterococcen* in the ileal digesta was reduced ($P < 0.05$).

Table 3. *Effect of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC) and rye/wheat on weights of the small intestine and caeca, and viscosity, pH and concentration of volatile fatty acids (VFA) of ileal digesta in broiler chicks at 22 d of age.*

	Dietary treatments				RSE
	corn	corn + 30 g HMC/kg	corn + 30 g LMC/kg	rye/ wheat	(df = 38)*
Weights (incl. contents) in % of BW of:					
Duodenum + jejunum	5.01^{a}	6.60^{d}	6.22^{c}	5.92^{b}	0.323
Ileum	2.31^{a}	3.56^{c}	2.95^{b}	2.96^{b}	0.212
Total small intestine	7.32^{a}	10.16^{c}	9.17^{b}	8.89^{b}	0.500
Caeca	0.78^{a}	0.87^{b}	0.75^{a}	0.87^{b}	0.104
pH	6.78^{a}	5.76^{d}	6.42^{b}	6.14^{c}	0.281
Digesta viscosity (cP[1])	2.61^{a}	nm	3.37^{b}	40.70^{c}	0.631
Fatty acids (mg/kg chymous)					
Total VFA	0.70^{b}	0.71^{b}	0.54^{a}	0.93^{c}	0.163
Acetic acid	0.37^{a}	0.35^{a}	0.26^{b}	0.25^{b}	0.076
Valeric acid	0.33^{a}	0.35^{a}	0.28^{a}	0.68^{b}	0.098

^{a,b,c,d} Mean values within a row with no common superscripts differ significant ($P \leq 0.05$).

nm = not measurable

* Except for viscosity (df = 28).

[1] cP = centiPoise (1 cP = 1/100 dyne sec/cm^2 = 1 mPa.s).

The addition of HMC, LMC or rye/wheat to the diet reduced significantly the concentration of conjugated bile acids in ileal digesta (Table 5). This reduction was observed for both chenodeoxycholic acid and cholic acid and occurred in birds fed diets containing all three NSP sources. The concentration of unconjugated bile acids was increased significantly by the inclusion in the diet of HMC or rye/wheat. The increase in concentration of unconjugated bile acids in the HMC fed birds was caused by an increase in both chenodeoxycholic acid and cholic acid, whereas in birds fed the rye/wheat-based diet only the concentration of unconjugated chenodeoxycholic acid was increased.

Table 4. *Effect of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC) and rye/wheat on the number of colony forming units of anaerobic and aerobic bacterial species (log cfu/g) in the ileal digesta of broiler chicks at 22 d of age.*

	Dietary treatments				RSE
	corn	corn + 30 g HMC/kg	corn + 30 g LMC/kg	rye/ wheat	(df = 38)
Total aerobic colony counts	7.4^{ab}	8.5^{c}	7.2^{a}	7.9^{b}	0.62
Total anaerobic colony counts	7.1^{a}	8.6^{c}	6.8^{a}	7.7^{b}	0.68
Bifidobacterium spp	6.1^{a}	7.5^{b}	6.0^{a}	6.7^{ab}	0.97
Lactobacillus spp	5.9^{ab}	6.7^{b}	5.2^{a}	6.5^{b}	1.11
Enterococcen spp	5.0^{b}	6.4^{c}	3.7^{a}	4.6^{ab}	1.33
Bacteroidaceae	3.4^{ab}	3.8^{b}	3.2^{a}	3.5^{ab}	0.69
Clostridium	6.1	6.4	6.2	6.4	0.68
E.coli	5.9^{a}	8.1^{c}	6.0^{a}	7.2^{b}	0.95

^{a,b,c} Mean values within a row with no common superscripts differ significant ($P \leq 0.05$).

Discussion

The results of the present study showed that the performance of broiler chicks was affected adversely when HMC, LMC or rye plus wheat were included in the diet. In accordance to previous studies (Langhout and Schutte, 1996; Langhout *et al.*, 1998), the present study showed that the anti-nutritive effects of citrus pectin in broiler chicks was dependent on the degree of methylation. The extent of the negative effects of both pectin products on performance was further influenced by the type of fat in the diet. The same was true when birds were fed the diet with rye in combination with wheat. Antoniou and Marquardt (1982) and Ward and Marquardt (1983) showed a similar interaction between the anti-nutritional effect of rye and the dietary fat source, using either tallow or soya oil. It is often hypothesized that the reduction in digestibility of nutrients and performance of chicks when feeding components containing water-soluble NSP is the result of an increment of the digesta viscosity. This increase in digesta viscosity may limit the mixing of nutrients with pancreatic enzymes and bile acids within the gastrointestinal tract (Edwards *et al.*, 1988), and also the movement of nutrients towards the gastrointestinal wall (Fengler and Marquardt, 1988). This will limit digestion and absorption. Results of the present study show that at similar digesta viscosity, differences in anti-nutritive effects of NSP on performance were found. Therefore, the results of the present study suggest that an increase in digesta viscosity is not the only factor that was responsible for the negative effects of water-soluble NSP on chick performance.

In the present study the inclusion of rye and wheat in the diet was associated with an increase in the concentration of VFA and a decrease in pH of ileal digesta. Both effects are associated with an increased microbial activity in the small intestine. The increased microbial activity in the small intestine of birds fed the rye/wheat diet is also is supported by the observed increase in ileal weight on the rye/wheat diets as part of this increase will be due to an increase in digesta content. Taking into account the increment in ileal weight of birds fed the HMC diet, it can be assumed that HMC also stimulated microbial activity. This is supported by the reduction in pH of the ileal digesta of birds fed the HMC diet. In addition, the addition of HMC to the diet increased the population of microorganisms in the small intestine. Our findings are in agreement with those reported by other investigators, who showed that the population of microorganisms was increased in the small intestine of chicks fed diets containing rye (Wagner and Thomas, 1978), wheat (Choct *et al.*, 1996) or HMC (Langhout *et al.*, 1998). These investigators suggested that excessive fermentation in the small intestine may limit normal physiological processes of nutrient digestion and absorption. It can be hypothesized that an increase in digesta viscosity slows down the hydrolysis of feedstuffs and absorption of nutrients. As a consequence, more undigested nutrients are present further down in the intestinal tract, which promotes microbial growth and consequently increases microbial activity.

The significance of gut microflora to the nutrition of chicks is not well documented. It is, however, well known that microbial degradation of nutrients is coupled with considerable losses in energy, not accounted in the ME determination, as compared to enzymatic hydrolysis and digestion of nutrients. In pigs these losses in energy are assumed to vary between 33% (ARC, 1981) and 50% (Just *et al.*, 1983; Van Es, 1987). No published data are available for poultry, but energy losses of a similar magnitude may be assumed. This is supported by the findings of several investigators (Hedge *et al.*, 1982; Muramatsu *et al.*, 1991), who reported that chicks can utilize energy extracted through the action of the gut bacteria, but the efficiency of its utilization is low. Besides the reduction in energy, the microflora can incorporate amino acids into microbial protein (Salter and Coates, 1974), which may lower N utilization as shown by Furuse and Yokota (1985).

Table 5. *Effect of high-methylated citrus pectin (HMC), low-methylated citrus pectin (LMC) and rye/wheat in the diet of 22 d old chicks on the content of conjugated and unconjugated bile acids (mmol/kg) in the ileal digesta.*

	Dietary treatments				RSE
	corn	corn + 30 g HMC/kg	corn + 30 g LMC/kg	rye/ wheat	(df = 38)
Conjugated bile acids					
Chenodeoxycholic acid	2.12^{a}	0.64^{b}	1.58^{a}	0.84^{b}	0.812
Cholic acid	0.79^{a}	0.07^{c}	0.43^{b}	0.13^{c}	0.324
Total	2.91^{a}	0.71^{c}	2.01^{b}	0.96^{c}	1.088
Unconjugated bile acids					
Chenodeoxycholic acid	1.17^{a}	2.78^{c}	1.54^{a}	2.25^{b}	0.473
Cholic acid	0.35^{a}	0.61^{b}	0.37^{a}	0.40^{a}	0.192
Total	1.52^{a}	3.39^{c}	1.90^{a}	2.69^{b}	0.578
Total bile acid concentration	4.43	4.10	3.91	3.65	1.150

^{a,b,c} Mean values within a row with no common superscripts differ significant ($P \leq 0.05$).

The increase in microbial activity in the small intestine in birds fed the HMC and rye/wheat based diets is probably also responsible for the corresponding increase in degree of bile acid deconjugation. Masclee *et al.* (1989) suggested that in humans determination of unconjugated bile acids is of value in the evaluation of bacterial overgrowth in the small intestine. Bile acids are required for the formation of emulsified particles, necessary for fat hydrolysis, and for the formation of micelles, necessary for absorption of fatty acids. According to Hylemond (1985), the intestinal bacteria are able to deconjugate bile acids. Unconjugated bile acids are rapidly absorbed from the small intestine of humans (Setchell *et al.*, 1982). However, reutilization of unconjugated bile acids from portal blood is less effective than that of conjugated bile acids. In humans 40 - 50% of the unconjugated bile acids are spilled over into the systemic circulation (Angelin *et al.*, 1982). A reduction in the recycling of bile acids may cause a decrease in the pool size (Juste *et al.*, 1983). Young chicks have a limited capacity to produce bile salts in the first few weeks after hatching (Green and Kellog, 1987; Iñarea *et al.*, 1989). As a result, the total concentration of bile acids in the intestinal digesta may be reduced. In the present study, however, the total concentration of bile acid in the ileal digesta of 22 d old birds was hardly affected by the diet. Birds at this age may therefore be able to produce sufficient quantities of bile acids for fat absorption (Green and Kellog, 1987; Iñarea *et al.*, 1989). The anti-nutritive effects of viscous water-soluble NSP on bile acid concentration will therefore be less pronounced in older as compared to younger birds. This agrees with the findings of Marquardt *et al.* (1979) and Veldman and Vahl (1994), who showed that the anti-nutritive effects of water-soluble NSP are more pronounced in young birds than in older birds.

An increase in microbial activity, due to the presence of water-soluble NSP in the diet, may also reduce the formation of micelles which may affect digestion of fat. It may be that a reduction in the formation of micelles is more critical for animal fat than for soya oil as it is more difficult to solubilize animal fat than soya oil. Moreover, Garrett and Young (1975) showed that short chain and unsaturated fatty acids can be more easily digested in the absence of micelle formation than long chain and saturated fatty acids. This observation agrees well with the results of the present study showing that the adverse effects of the viscous water-soluble NSP on chick performance was more pronounced when the diet contained blended animal fat instead of soya oil.

Furthermore, the present study indicates that inclusion of HMC in the diet not only increased microbial activity, but also changed the balance of microorganisms in the gut ecosystem. Addition of HMC to the diet raised the number of *Bifodobacteria*, *Enterococci* and *E.coli*. In birds fed the rye/wheat containing diet only the number of *E.coli* increased, whereas inclusion of LMC in the diet had hardly any effect on microbial composition. Similar changes in the composition of the microflora in the small intestine by feeding chicks diets containing HMC were observed in a previous study (Langhout *et al.*, 1998). It is not known, however, if the activity of the microflora in the small intestine further modifies the magnitude of the anti-nutritive effects of viscous water-soluble NSP in chicks. Studies with abiotic chicks would provide information relative to this effect.

The results of the present study suggest that the type and level of microbial activity in the small intestine in addition to digesta viscosity are collectively responsible for the anti-nutritive effects of water-soluble NSP on the chick performance. The water-soluble NSP through their effects of the microbial population may also increase deconjugation of bile acids. This increase in bile acid deconjugation may play a role in the interaction between dietary fat source and the anti-nutritive effects of water-soluble NSP. Further studies are required to determine the exact effect of bile acid deconjugation on fat digestion and absorption, and to clarify the role of the microflora on the anti-nutritional effects of viscous carbohydrates.

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References

Agricultural Research Council (1981) The nutrient requirement of pigs. Slough: Commonwealth Agricultural Bureaux.

Angelin, B., Bjorkhem, I., Einarsson, K. & Ewerth, S. (1982) Hepatic uptake of bile acids in man. *Journal of Clinical Investigation* 70: 724-731.

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

Antoniou, T., Marquardt, R.R. & Misir, R. (1980) Utilization of rye diets by chicks as affected by lipid type and level and penicillin supplementation. *Poultry Science* 59: 758-769.

Antoniou, T., Marquardt, R.R. & Cansfield, P.S. (1981) Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agricultural Food and Chemistry* 28: 1240-1247.

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens; effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. *British Poultry Science* 24: 191-203.

Choct, M. & Annison, G. (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Moran, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Coates, M.E., Cole, C.B., Fuller, R., Houghton, S.B. & Yokota, H. (1981) The gut microflora and the uptake of glucose from the small intestine of the chicks. *British Poultry Science* 22: 289-294.

Dutch Bureau of Livestock Feedingstuffs (1995) Chemical composition, digestibility and Energy value of feed ingredients. Bureau of Livestock Feedingstuffs, Lelystad, The Netherlands.

Edwards, C.A., Johnson, I.I. & Read, N.W. (1988) Do viscous polysaccharides slow absorption by inhibiting diffusion or convection. *European Journal of Clinical Nutrition* 42: 307-312.

Faassen, van A., Nagengast, F.M., Hectors, M., Van den Broek, W.J.M. Huijbregts, A.W.M., Van der Werf, S.D.J., Berge Henegouwen, G.P. & Van Tongeren, J.H.M. (1985) Determination of individual human faecal bile acids by gas- liquid chromatochraphy after enzymatic deconjugation and simultaneous solvolysis and methylation using dimethoxypropane. *Clinica Chimica Acta* 152: 231-239.

Fengler, A.I. & Marquardt, R.R. (1988) Water-soluble pentosans from rye: II effects on rate of dialysis and on retention of nutrients by the chick. *Cereal Chemistry* 65: 298-302.

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

Furuse, M. & Yokota, H. (1985) Effect of the gut microflora on chick growth and utilization of protein and energy at different concentrations of dietary protein. *British Poultry Science* 26: 97-104.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Green, J. & Kelloch, T.F. (1987) Bile acid concentration in serum, bile, jejunal contents, and excreta of male broiler chicks during the first six weeks posthatch. *Poultry Science* 66: 535-540.

Hedge, S.N., Rolls, B.A. & Coates, M.E. (1982) The effects of the gut microflora and dietary fibre on energy utilization by the chick. *British Journal of Nutrition* 48: 73-80.

Hylemond, P.B. (1985) Metabolism of bile acids in intestinal microflora. In: *Sterols and bile acids: New Comprehensive Biochemistry* (Danielsen, H. & Sjövall, J., eds.) Elsevier Science Publishers B.V. pp 331-343.

Iñarrea, P., Simon, M., Manzano, M. & Palacios, J. (1989) Changes in the concentration and composition of biliary and serum bile acids in the young domestic fowl. *British Poultry Science* 30: 353-359.

Just, A., Fernande, J.A. & Jorgensen, H. (1983) The net energy value of diets for growth in pigs in relation to the fermentative processes in the digestive tract and the site of absorption of the nutrients. *Livestock Production Science* 10: 171-186.

Juste, C., Demarne, Y. & Corring, T. (1983) Response of bile flow, biliary lipids and bile acid pool in the pig to quantitative variations in dietary fat. *Journal of Nutrition* 113: 1671-1701.

Langhout, D.J. & Schutte, J.B. (1996) Nutritional implications of pectins in chicks in relation to esterification and origin of pectins. *Poultry Science* 75: 1236-1242.

Langhout, D.J., Schutte, J.B., Van Leeuwen, P., Wiebenga, J. & Tamminga, S. (1998) Effect of dietary high- and low-methylated citrus pectin on activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *British Poultry Science* (in press).

Marquardt, R.R., Ward, A.T. & Misir, R. (1979) The retention of nutrients by chicks fed rye diets supplemented with amino acids and penicillin. *Poultry Science* 58: 631-640.

Masclee, A., Tangerman, A., Van Schaik, A., Van der Hoek, E.W. & Van Tongeren, H.M. (1989) Unconjugated serum bile acids as a marker of small intestinal bacterial overgrowth. *European Journal of Clinical Investigation* 19: 384-389.

Muramatsu, T., Kodama, H., Morishita, T., Furuse, M. & Okumura, J. (1991) Effect of intestinal microflora on digestible energy and fiber digestion in chickens fed a high-fiber diet. *American Journal of Veterinary Research* 52: 1178-1181.

National Research Council (1994) Nutrient requirements of poultry, 9th revised edn. (Wasington DC, National Academy of Sciences).

Norusis, M.J. (1992) SPSS/PC+ Base System User's Guide Version 5.0 (SPSS nc., Chicago, Illinois).

Princen, H.M.G., Meijer, P. & Kuipers, F. (1990) One step solvolysis of 3-, 7- and 12 sulphated free and conjugated bile acids. *Clinica Chimica Acta* 192: 77-84.

Salemans, J.M.J., Nagengast, F.M., Tangermen, A., Van Schaik, A., Hopman, W.P.M., De Haan, A.F.J. & Jansen, J.B.M.J. (1993) Effect of ageing on postprandial conjugated and unconjugated serum bile acid levels in healthy subjects. *European Journal of Clinical Investigation* 23: 192-198.

Salter, D.N. & Coates, M.E. (1974) The utilization of protein and excretion of uric acid in germ-free and conventional chicks. *British Journal of Nutrition* 31: 307-318.

Setchell, K.D.R., Lawson, A.M., Blackstock, E.J. & Murphy, G.M. (1982) Diurnal changes in serum unconjugated bile acids in normal man. *Gut* 23: 637-642.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical methods. 7th ed. (The Iowa State University Press. Ames, 1A).

Smits, C.H.M. (1996) Viscosity of dietary fibre in relation to lipid digestibility in broiler chicks. PhD thesis, Agricultural University Wageningen, the Netherlands.

Tangerman, A., Van Schaik, A. & Van der Hoek, W. (1986) Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects. *Clinica Chimica Acta* 159: 123-132.

Van Es, A.J.H. (1974) Energy utilization of low digestibility carbohydrates. In: *Low digestibility carbohydrates* (Leegwater, D.C., Feron, V.J. & Hermus, R.J.J., eds.). Wageningen: Pudoc Press, pp. 121-127

Veldman, A. & Vahl, H.A. (1994). Xylanase in broiler diets with differences in characteristics and content of wheat. *British Poultry Science* 35: 537-550.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

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

Chapter 6

Effect of viscosity on digestion of nutrients in conventional and germ-free chicks

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Abstract

A study was conducted with conventional and germ-free broiler chicks to obtain more information on the role of the intestinal microflora on the anti-nutritive effects of non-starch polysaccharides (NSP) in broiler chicks. The three diets used, contained 500 g maize/kg, 500 g maize and 30 g highly methylated *citrus* pectin (HMC)/kg and 300 g rye/kg, respectively. Feeding the HMC or the rye diet to conventional birds depressed weight gain and food utilization ($P < 0.05$), whereas in germ-free birds only weight gain was reduced ($P < 0.05$) when HMC was included in the diet. Feeding the HMC diet to conventional birds reduced digestibility of energy and starch at the end of the jejunum. Ileal digestibility of starch and energy were not strongly affected when birds were fed the HMC-containing diet. Faecal digestibility of organic matter, crude fat, starch and amino acids, nitrogen retention and metabolizable energy were reduced when conventional chicks were fed the HMC diet. Feeding the HMC diet to germ-free birds hardly affected faecal digestibility of organic matter, crude fat, starch and amino acids and nitrogen retention, whereas metabolizable energy was increased. Feeding the HMC or the rye diet to conventional or germ-free birds increased viscosity of digesta in the small intestine. This increase in digesta viscosity was more pronounced in conventional than in germ-free birds. The pH of ileal digesta was reduced when HMC or rye was added to the diet of conventional chicks, but not in germ-free chicks. Feeding the HMC or the rye diet to conventional birds markedly affected morphology of the gut wall, whereas in germ-free chicks only little effect was found on gut morphology. Based on the results of the present study, it was concluded that the gastrointestinal microflora mediates the magnitude of the anti-nutritive effects of water-soluble NSP in broiler chicks by affecting viscous properties and fermentation of water-soluble NSP in the gastrointestinal tract.

Introduction

Water-soluble non-starch polysaccharides (NSP) such as pentosans in wheat and rye are held responsible for the reduction in performance and nutrient digestibility of broiler chicks (White et al., 1981; Fengler and Marquardt, 1988; Choct and Annison, 1990; 1992). The mechanism by which these NSP fractions exert their anti-nutritive activities is still not well understood. It is assumed that an increase in viscosity of the aqueous fraction, as a result of their viscous properties, is the primary mechanism by which these water-soluble NSP reduce nutrient digestibility (Fengler and Marquardt, 1988; Choct and Annison, 1992). An increase in viscosity of digesta may limit mixing of nutrients with pancreatic enzymes and bile acids (Edwards et al., 1988). Moreover, movement of nutrients towards the gastrointestinal wall is reduced by an increment of digesta viscosity, which consequently limits digestion and absorption (Antoniou et al., 1981; Fengler and Marquardt, 1988).

Several studies indicate that the magnitude of the anti-nutritive effect of viscous water-soluble NSP is affected by dietary fat (Antoniou and Marquardt, 1982; Ward and Marquardt, 1983; Langhout et al., 1998[b]). These investigators found that the magnitude of the anti-nutritive effects were more pronounced when tallow or blended animal fat instead of soya oil was included in the diet. The increase in ileal viscosity, due to water-soluble NSP in the diet, however, was found not to be affected by the type of fat (Langhout et al., 1998[b]). These results suggest that the increase in digesta viscosity alone cannot explain the anti-nutritive effects of the water-soluble NSP. Choct et al. (1992) indicate that an increased flow of undigested nutrients to the end of the small intestine, as a result of the increased digesta viscosity, promotes microbial growth. This is supported by data of Wagner and Thomas (1978) and Langhout et al. (1998[a,b]), who showed microbial activity to be elevated in the ileum of birds fed diets containing rye or highly methylated citrus pectin (HMC). An increment in microbial activity in the intestinal tract increases deconjugation of bile acids in the small intestine as shown by Langhout et al. (1998[b]). This reduction in conjugated bile acids was suggested to reduce the formation of micelles, which may limit absorption of fatty acids (Coates et al., 1981). These results indicate that, in addition to viscosity, the intestinal microflora influenced the magnitude of the anti-nutritive effects of water-soluble NSP in broiler chicks.

The present study was undertaken to obtain more information on the role of the intestinal microflora on the anti-nutritive properties of water-soluble NSP by using conventional and germ-free broiler chicks. The water-soluble NSP containing products involved in the study were HMC and rye.

Materials and methods

Diets

The composition of the basal diets is presented in Table 1. Both basal diets were formulated to meet the requirements of broiler chicks (National Research Council, 1994). Additives such as antibiotics or coccidiostats were omitted from the diets, as they may interfere with microbial activity. Highly methylated citrus pectin (HMC; type CU 301, 30 mg/kg, methylation >65%, Contined, Bennekom, The Netherlands) was added to the maize-based diet. The pectin product, which was supplied as anhydrous polysaccharide, was substituted in the diet by weight for Diamol (5 g/kg), an insoluble ash (Franz Bertram, Hamburg, Germany) and tapioca (25 g/kg). The ME value for both HMC and Diamol was assumed to be zero.

Animals

In the trial with conventional birds one-day-old male broiler chicks ("Ross") were used, which were housed in two-tiered, electrically heated battery cages having wire floors with a floor space of 975 cm^2. The cages were located in an insulated room of controlled temperature and humidity. Chicks were subjected to continuous artificial fluorescent illumination. Each treatment group was allotted at random to cages with 24 birds each. The experimental diets were fed for 21 d (1 to 21 d of age). Chicks were weighed individually and food consumption for each cage was recorded at the end of the trial. Water intake was measured for each cage separately at intervals of 12 h for 4 d (15 to 19 d of age) as the difference between a predetermined volume of water in the trough and the volume remaining in the trough. The diets (as pellets) were fed *ad libitum*. Water was available *ad libitum* via an automatic device.

The trial with germ-free chicks consisted of two sub-trials. Each sub-trial was carried out under similar conditions. Naturally clean 18-d-old brood eggs of a normal broiler strain ("Ross") were incubated, sterilized with formaldehyde (37%) for 20 min, injected with 0.33 ml Exenal per egg (natrium ceftiofur; Upjohn, USA) and hatched in a pre-isolator equipped with thermostatically controlled supplementary heat sources. Two days after hatching, birds were sexed and transferred to one of the six isolators.

Table 1. *Composition of the basal diets in g/kg.*

Ingredient	Maize-based	Rye-based
Maize	500.0	200.0
Rye	-	300.0
Tapioca	27.0	55.6
Soya oil	25.0	30.0
Animal fat	60.0	60.0
Soya bean meal (500 g/kg CP)	256.0	240.0
Casein	63.0	52.0
Fish meal	20.4	20.0
Diamol	5.0	-
Limestone	13.3	12.1
$Ca(H_2PO_4)_2$	13.4	13.2
Salt	2.3	2.0
Vitamins + minerals mixture[1]	10.0	10.0
$NaHCO_3$	1.8	2.0
$KHCO_3$	1.6	1.0
DL-methionine	1.2	1.4
L-arginine	-	0.7
Contents:		
Dry matter (analysed, g/kg)	893	894
Crude protein (analysed, g/kg)	228	222
Crude fat (analysed, g/kg)	98	97
Ash (analysed, g/kg)	72	58
Starch (analysed, g/kg)	326	310
Metabolizable energy (calculated, MJ/kg)[2]	12.8	12.2
Ca (calculated, g/kg)	8.7	8.6
P (calculated, g/kg)	7.0	6.9
Lysine (analysed, g/kg)	13.8	13.2
Methionine + cystine (analysed, g/kg)	9.0	9.2

[1] Supplied per kilogram of diet: riboflavin, 4 mg; nicotinamide, 40 mg; d-pantothenic acid,12 mg; choline chloride, 500 mg; cyanocobalamin, 15 µg; Dl-α-tocopheryl acetate, 20 mg; menadione, 5 mg; retinyl acetate, 3.44 mg; cholecalciferol, 50 mg; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4.7H_2O$, 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$, 100 mg; $ZnSO_4.H2O$, 150 mg; $Na_2SeO_3.5H_2O$, 0.15 mg; KI, 5 mg; $CoSO_4.7H_2O$, 1 mg; and antioxidant (ethoxyquin), 100 mg.

[2] Calculated from the data provided by the Dutch Bureau of Livestock Feeds (1994).

To each isolator 5 female and 5 male chicks were allotted such that the average weight of the birds in each isolator was similar (77 g). Thus, in total each group consisted of 4 isolators with 5 female and 5 male birds each. The isolators as well as the materials entering or leaving the isolator were sterilized with a formaldehyde (37%) spray. All diets were sealed in plastic bags and sterilized by irradiation at 20 kRay (Gammaster, Ede, Netherlands). Feed (as pellets) and water were available ad libitum. Birds were fed the maize-based diet before they were transferred to the isolators. Excreta samples were taken every week to check on microbial contamination. In the first trial, birds were not contaminated with any bacteria in the intestinal tract. In the second sub-trial, birds were contaminated to some extent with *Bacillus lichenifermis.* To minimize growth of *B. lichenifermis* drinking water was medicated with 4 ml/L Methoxasol-T (trimethoprim 20 mg/ml + sulfamethoxazol 100 mg/ml; Vetimex Animal Health BV, Cuyck, the Netherlands).

The experimental diets were fed for 18 d (3 to 21 d of age). Chicks were weighed individually and food consumption for each cage was recorded at the end of the trial. Water consumption was measured for each isolator separately during the collection period as the difference between a predetermined volume of water in the trough and the volume remaining in the trough.

Digestibility

In both trials digestibility of the nutrients and metabolizable energy of the maize diet and the maize diet with HMC was determined. This was done during the age period of 15 to 19 days. During this 4 d period the excreta were collected quantitatively according to the procedure described by Langhout et al. (1998[a]). In the trial with conventional birds excreta were collected at 4 h intervals during the day (08:00-20:00), and once for the night (20:00-08:00). Day and night excreta were collected separately. Only the excreta collected during the day were used for analyses. In the trial with germ-free birds all excreta produced during the 4 d collection period were used for analyses. In both trials excreta were collected for each cage separately. In the trial with conventional chicks 4 cages/treatment were involved in the digestibility study, and in the trial with germ-free birds all isolators. The excreta were stored in closed containers at - 20 °C.

At the end of the trials, both diets and the excreta were analysed for dry matter, nitrogen, fat, starch, ash, amino acids and gross energy (GE). In addition, the dry matter content of the night excreta was determined for calculating total dry matter output per 24 h. The AME of each diet was calculated from the figures for GE of the food and excreta. The AME values were corrected to zero nitrogen balance (AME_n). The correction factor used was 34.39 kJ per gram retained nitrogen as proposed by Hill and Anderson (1958).

Samples of feed and freeze-dried excreta were milled to pass through a 1.0 mm screen before analysis. All analyses were carried out in duplicate. Dry matter content was determined by drying the samples to a constant weight at 101°C. Inorganic matter and N were determined by standard methods (Association of Official Analytical Chemists, 1984), GE was determined with an IKA-C4000 adiabatic colorimeter. Crude fat was determined by treating feed or excreta for 1 h with 4 M HCl and drying for 3 h under vacuum at 100°C, followed by 8 h extraction with hexane. Starch content was determined enzymatically by hydrolysing the starch to glucose with amyloglucosidase (NEN 3574). Amino acids were determined by ion-exchange chromatography (Slump, 1969) after hydrolysis of the samples with 6 M HCl for 24 h at 100 C. Methionine and cystine were determined by ion-exchange chromatography as methionine sulfate and cysteic acid, respectively, after oxidation with performic acid. Tryptophan was not determined.

Additional measurements

After completion of the performance trial, fifteen randomly selected conventional birds per cage originating from the cages in which also faecal digestibility of nutrients was determined (= 60 birds per treatment group) were used for determination ileal digestibility. Food and water remained available to these birds until sacrifice at 22 d of age, after which they were weighed individually. After sacrifice through injection of T61 (Embutramide-Mebezoniumiodide-Tetracainhydrochloride mix, Hoechst, Germany), samples were taken of the jejunum 10 cm preceding Meckel's diverticulum and of the ileum 10 cm preceding the ileo-caecal junction, for measuring jejunal and ileal digestibility of energy and starch as described by Van der Klis et al. (1993). The jejunal and ileal samples were analysed in pooled samples of 15 chicks each taken from a different cage.

From all cages, 4 randomly selected conventional birds per cage (= 24 birds per treatment group) as well as all germ-free birds (= 40 per treatment group) were used for additional measurements. Food and water remained available to these birds until sacrifice at 22 d of age, after which they were weighed individually. After sacrifice through injection of T61, the small intestine was removed immediately and the weight of the duodenum plus jejunum and ileum was recorded. In addition, caecal weight including their contents was determined, and samples 3 cm long were taken from the intestinal wall, 15 cm distal of Meckel's diverticulum, for assessing morphological characteristics. For these measurements two birds per cage were taken. In addition, samples of the digesta of the duodenum plus jejunum and the ileum were taken for measuring viscosity and pH. In the samples of ileal digesta of the conventional birds, lactic acid, formic acid and volatile fatty acids (VFA) were also determined. The samples were taken from the beginning of the duodenum to Meckel's diverticulum and from Meckel's diverticulum to the end of the small intestine. Analyses in the duodenum plus jejunum and ileal digesta were performed in pooled samples of four chicks each taken from a different cage or in one pooled sample per isolator.

Analytical methods

Ileal viscosity. The viscous property of the intestinal digesta was determined with a Brookfield digital viscometry (Model LVTD VCP-II, Brookfield Engineering Laboratories, USA) according to the method of Bedford and Classen (1993).

The *pH*. The pH of the aqueous fraction was measured in the same sample in which ileal viscosity was determined. The pH was measured by inserting a micro pH electrode (LoT 440-M3, Dr. W. Ingold, Udorf, Germany) into the aqueous fraction.

VFA. Concentrations of lactic acid, formic acid and VFA (acetic, propionic, isobutyric, butyric, iso-valeric and valeric acids) of the ileal digesta was determined by the gas-liquid chromatography method (GLC; model HP 5890, Hewlett, Palo Alto, CA) of Langhout and Schutte (1996).

Morphological characteristics. Each sample of the small intestine was cut open longitudinally at the antimesenteric attachment. Samples were fixed on dental wax with the villi on the upper side and fixed in 0.1 M-phosphate-buffered formalin solution (40 ml/l). The shape of the villi was studied with a dissecting microscope and characterized as described previously (Langhout et al., 1998[a]). The following classes of villi were distinguished: tongue-shaped, finger-shaped, leaf-shaped, ridge-shaped and convoluted villi.

Statistical analysis

Data were subjected to analysis of variance with the SPSS/PC + V5.0 computer program of Norusis (1992). Treatment factors were type of diet and battery tier for the trial with conventional chicks and type of diet and sub-trial for the trial with germ-free chicks. The treatment means were tested for difference by using the Least Significant Difference test (Snedecor and Cochran, 1980). All statements of significance are based on a probability of less than 0.05.

Weight gain, food intake, food conversion efficiency, water consumption, faecal digestibility of nutrients, nitrogen retention, metabolizable energy content, weights of the small intestine and caeca, pH and viscosity of the ileal digesta for the germ-free birds in both sub-trials were similar and statistically not significant. This indicates that the contamination of the birds with *B. licheniformis* in the second sub-trial did not interfere with the results of the trial.

Table 2. *Effect of highly methylated citrus pectin (HMC) and rye on performance in conventional (1 to 21 d of age) and germ-free (3 to 21 d of age) chicks.*

	Dietary treatment			SEM
	maize	maize + 30 g HMC/kg	rye	
Conventional chicks				(df=20)
Weight gain (g)	773^{c}	696^{a}	733^{b}	6.4
Food intake (g/d per bird)	48.8^{a}	50.9^{b}	52.2^{c}	0.29
Food utilization (MJ per kg gain)	17.04^{a}	19.73^{c}	18.17^{b}	0.102
Water intake (g/d per bird)[1]	139^{a}	181^{b}	180^{b}	2.7
Water/food ratio[1]	1.83^{a}	2.32^{c}	2.14^{b}	0.028
Germ-free chicks				(df = 8)
Weight gain (g)	737^{b}	694^{a}	709^{ab}	11.2
Food intake (g/d per bird)	51.1^{ab}	49.5^{a}	52.2^{b}	0.80
Food utilisation (MJ per kg gain)	16.95	17.44	17.00	0.152
Water intake (g/d per bird)[1]	154	154	168	4.7
Water/food ratio[1]	2.03^{a}	2.28^{b}	2.26^{b}	0.074

[1] Means of 4 d (age period 17 - 21 d).

[a,b] Mean values within a row with no common superscript differ significantly ($P<0.05$).

Results

The mortality rate was low as only 1.5% of the conventional birds and 1.7% of the germ-free birds died. The performance data of both trials are summarized in Table 2. In both trials, treatment groups did not differ appreciably in mortality rate. Feeding the HMC diet to conventional chicks depressed weight gain and food utilization by

9.5% and 15.2%, respectively. Inclusion of rye in the diet of the conventional birds, resulted also in less well results for weight gain (5%; $P < 0.05$) and food utilization (6.4%; $P < 0.05$) compared to those fed the control diet. In germ-free chicks fed on the HMC diet only, weight gain was reduced significantly by 5.8%, whereas food utilization decreased only slightly ($P > 0.05$). Germ-free birds fed the rye-based diet produced almost similar results for weight gain and food utilization as those fed the maize-based diet. Feeding the HMC or the rye diet to conventional birds resulted in an increased water intake and water/food ratio ($P < 0.05$). Feeding these diets to germ-free birds resulted in an elevated water/food ratio ($P < 0.05$).

Table 3. *Effect of highly methylated citrus pectin (HMC) on jejunal and ileal digestibility coefficients of starch and energy in conventional chicks.*

	Dietary treatment		SEM
	maize	maize + 30 g HMC/kg	(df=5)
Digestibility at the end of the jejunum			
- energy	65.2^a	51.5^b	1.81
- starch	89.4^a	82.9^b	1.41
Digestibility at the end of the ileum			
- energy	72.5^a	65.2^b	0.78
- starch	95.6^a	92.6^b	0.44
Digestibility in the ileum[1]			
- energy	20.9	28.2	4.45
- starch	57.5	56.7	4.53

[1] Calculated as (ileal digestible nutrient - jejunal digestible nutrient)/(100 - jejunal digestible nutrient).

a,b Mean values within a row with no common superscripts differ significantly (*P*<0.05).

Feeding the HMC diet to conventional chicks reduced digestibility of energy and starch at the end of the jejunum (Table 3). At the end of the ileum, digestibility of energy and starch was only slightly affected by the HMC diet.

HMC reduced faecal digestibility of dry matter, organic matter, crude fat, nitrogen retention and metabolizable energy value of the diet in conventional chicks (Table 4). Feeding the HMC diet to germ-free chicks, however, had only little effect on faecal digestibility of dry matter, organic matter, crude fat and nitrogen retention ($P > 0.05$), whereas that diet's metabolizable energy value increased ($P < 0.05$; Table 4). Digestibility of amino acids was significantly depressed when HMC was added to the diet of conventional chicks (Table 5). Digestibility of the individual amino acids declined between 17.5 and 7.2% in the conventional chicks fed the HMC diet. In germ-free chicks hardly any effect on the digestibility of amino acids was found.

Table 4. *Effect of highly methylated citrus pectin on faecal digestibility, nitrogen retention and metabolizable energy in conventional and germ-free chicks*

	Dietary treatment		SEM
	maize	maize + 30 g HMC/kg	(df=5)
Conventional chicks			
- dry matter	71.6^{b}	64.8^{a}	0.25
- organic matter	73.3^{b}	68.8^{a}	0.26
- crude fat	81.3^{b}	65.7^{a}	0.66
- starch	96.9^{b}	95.3^{a}	0.11
Nitrogen retention (% of intake)	56.9^{b}	51.9^{a}	0.33
Metabolizable energy (ME_n, MJ/kg)	13.32^{b}	11.81^{a}	0.071
Germ-free chicks			
- dry matter	74.4	74.6	0.70
- organic matter	76.7	76.4	0.65
- crude fat	93.9	94.8	0.32
- starch	98.9	98.9	0.15
Nitrogen retention (% of intake)	62.4	63.6	1.24
Metabolizable energy (ME_n, MJ/kg)	13.76^{a}	14.26^{b}	0.095

^{a,b} Mean values within a row with no common superscripts differ significantly ($P<0.05$).

Feeding the HMC or the rye diet to conventional chicks increased viscosity of digesta in the proximal part of the small intestine compared to birds fed the control diet ($P < 0.05$; Table 6). The highest value for digesta viscosity was measured in birds fed the HMC diet. Digesta viscosity in the ileum of conventional birds fed the rye-based diet was also increased compared to the ileal viscosity figures found in birds fed the control diet ($P < 0.05$). Digesta of the ileum content of birds fed on the HMC diet was gel-like and had a high water-binding capacity. Digesta viscosity of birds fed this diet could not be measured as no supernatant was obtained after centrifugation of the digesta (10,000×*g* for 10 min at 4 °C), but was certainly very high. In germ-free chicks, viscosity of digesta in the proximal and the distal part of the small intestine was also elevated when HMC or rye was included in the diet, being more pronounced for the HMC-fed birds. However, the magnitude of the increase in digesta viscosity was markedly lower than in conventional chicks. Feeding the HMC or the rye diet to conventional chicks had little effect on digesta pH in the proximal part of the small intestine, whereas the pH in the ileal digesta was reduced significantly (Table 6). Feeding the HMC or the rye diet to germ-free chicks had little effect on pH of digesta in the different parts of the small intestine. In germ-free birds higher values were found in the proximal and distal part of the small intestine than in conventional birds. This difference in pH value between germ-free and conventional birds was most pronounced in the HMC diet. In conventional birds fed the HMC or the rye diet, weights of the duodenum plus jejunum and of the ileum were higher than in birds fed the control diet (Table 6). Caecal weights tended to be higher in conventional birds fed either the HMC or the rye-based diet ($P > 0.05$). The inclusion of HMC or rye in the diet of germ-free chicks hardly affected the weight of the different parts of the small intestine, whereas caecal weights were increased significantly.

Feeding the HMC diet to conventional chicks increased the concentration of lactic acid in ileal digesta, whereas rye only slightly increased the concentration of lactic acid ($P > 0.05$; Table 7). The concentration of VFA in the ileal digesta was hardly affected by HMC or rye.

Feeding the HMC or the rye diet to conventional chicks affected the classification and the shape of the villi (Table 8). The number of zigzag patterns and ridge-shaped villi was reduced in conventional birds fed the HMC or the rye diet compared to those present in birds fed the control diet, whereas the number of tongue-shaped villi was higher. This effect was more pronounced for the HMC diet than for the rye diet. No significant change in classification and shape of the villi was observed when HMC or rye was included in the diet of germ-free chicks.

Table 5. *Effect of highly methylated citrus pectin (HMC) on faecal amino acid digestibility in conventional and germ-free chicks.*

	Dietary treatment		SEM
	maize	maize + 30 g HMC/kg	(df=5)
Conventional chicks			
Isoleucine	88.2^{b}	76.5^{a}	0.19
Leucine	89.7^{b}	77.4^{a}	0.24
Lysine	89.7^{b}	80.1^{a}	0.24
Methionine	92.1^{b}	82.2^{a}	0.2
Cystine	70.9^{b}	53.4^{a}	0.25
Methionine + cystine	84.5^{b}	72.1^{a}	0.21
Threonine	82.5^{b}	68.7^{a}	0.2
Valine	87.3^{b}	75.3^{a}	0.21
Arginine	91.7^{b}	81.7^{a}	0.13
Alanine	84.2^{b}	70.5^{a}	0.29
Aspartic acid	86.0^{b}	78.8^{a}	0.28
Glutamic acid	90.9^{b}	81.3^{a}	0.14
Glycine	77.8^{b}	64.1^{a}	0.16
Serine	86.2^{b}	74.2^{a}	0.2
Mean amino acids	85.9	73.8	--
Germ-free chicks			
Isoleucine	92.8	92.8	0.25
Leucine	94.1	94.2	0.22
Lysine	91	91.6	0.28
Methionine	94.3	94.4	0.24
Cystine	70.2	69.4	0.6
Methionine + cystine	86.2	85.3	0.31
Threonine	87.6	87.3	0.3
Valine	92	92.5	0.27
Arginine	93.3	93.5	0.22
Alanine	90.4	90.7	0.27
Aspartic acid	88.9	89.1	0.31
Glutamic acid	93.4	93.4	0.21
Glycine	82.4	82.6	0.48
Serine	89.1	88.8	0.31
Mean amino acids	91.2	91.1	--

[a,b] Mean values within a row with no common superscripts differ significantly ($P<0.05$).

Discussion

In the present study the hypothesis was tested whether an increase in microbial activity in the small intestine plays a role in the magnitude of the anti-nutritive properties of water-soluble NSP in broiler chicks. The results of the present study show that dietary addition of HMC reduces performance of conventional broiler chicks. The same was true when rye was included in the diet. These results agree well with those obtained in previous studies (Langhout and Schutte, 1996; Langhout et al, 1998[ab]). The reduction in performance of conventional birds fed HMC was at least partly due to a decrease in nutrient digestibility. The inclusion of HMC in the diet of germ-free chicks had only limited anti-nutritive effects on performance and nutrient digestibility compared to those observed in conventional chicks. The same held for performance when rye was included in the diet. Thus, our results indicate that the gastrointestinal microflora plays an important role in the magnitude of the anti-nutritive effects of water-soluble NSP in broiler chicks. Similar findings have been reported for rye by Campbell et al. (1983) who also showed that the decrease in performance was more pronounced in conventional chicks than in germ-free chicks.

The results of the present study show that inclusion of HMC to the diet of germ-free birds increased metabolizable energy of the diet. This increase in energy content of the diet was not reflected in the nutrient digestibility. On both diets, the maize and the pectin supplemented diet, the same results for digestibility of the nutrients were found. No possible explanation for the increase in metabolizable energy content when germ-free birds were fed the HMC diet can be given.

It is generally assumed that the viscous properties of water-soluble NSP are mainly responsible for the anti-nutritive effects in broiler chicks. In germ-free chicks the increase in digesta viscosity caused by diets containing HMC or rye was considerably lower than in conventional chicks fed the same diets. The effect of HMC and rye on water/food ratio was similar in conventional and germ-free birds. Therefore, the difference in digesta viscosity between germ-free and conventional chicks fed the HMC- or rye-based diets could not be explained by a difference in water consumption. It is, therefore, highly probable that the water-soluble NSP fractions are partly fermented by microorganisms into smaller fractions having stronger viscous properties. Ileal viscosity will thus increase and, as a result, may impair diffusion of pancreatic enzymes with nutrients (Edwards et al., 1988). Moreover, transport and mixing of digestive enzymes and nutrients may be limited,

Table 6. *Effect of highly methylated citrus pectin (HMC) and rye on digesta viscosity and pH of the proximal and distal part of the small intestine and on the weights of the small intestine and caeca of conventional and germ-free chicks at 22 d of age.*

	Dietary treatment			
	maize	maize + 30 g HMC/kg	rye	SEM
Conventional chicks				(df=20)^{2}
Digesta viscosity (cP^{1})				
Duodenum + jejunum	1.7^{a}	60.8^{c}	10.6^{b}	0.35
Ileum	2.4^{a}	n.m.	33.5^{b}	0.081
pH				
Duodenum + jejunum	5.3	5.2	5.3	0.14
Ileum	6.6^{a}	5.5^{b}	5.9^{b}	0.184
Weight (including contents) in % of BW				
Duodenum + jejunum	4.6^{a}	5.8^{c}	5.3^{b}	0.09
Ileum	2.0^{a}	3.0^{c}	2.4^{b}	0.06
Total small intestine	6.6^{a}	8.8^{c}	7.8^{b}	0.13
Caeca	0.79	0.89	0.84	0.04
Germ-free chicks				(df = 8)
Digesta viscosity (cP^{1})				
Duodenum + jejunum	2.2^{a}	7.3^{c}	5.2^{b}	0.39
Ileum	2.8^{a}	12.4^{c}	7.1^{b}	1.15
pH				
Duodenum + jejunum	6.6	6.5	6.6	0.054
Ileum	7.9	7.8	7.8	0.029
Weight (including contents) in % of BW				
Duodenum + jejunum	3.3	3.5	3.5	0.14
Ileum	1.6	1.9	1.7	0.10
Total small intestine	4.9	5.3	5.2	0.23
Caeca	0.81^{a}	1.62^{b}	1.27^{b}	0.122

n.m.: not measurable

[1] cP = centipoise (1 cP = 0.01 dyne s/cm^{2} = 1 mPa.s)

[2] Except for viscosity in the ileum (df = 13).

[a,b,c] Mean values within a row with no common superscripts differ significantly (*P*<0.05).

which reduces hydrolysis of nutrients by digestive enzymes (Fengler and Marquardt, 1988). This may slow down the digestion of nutrients in the proximal part of the small intestine. This notion is supported by the results of the present study, in which less energy and starch were digested at the end of the jejunum in conventional chicks fed the HMC diet than in chicks fed the maize diet. The increase in undigested nutrients in the lower part of the intestinal tract may promote microbial growth.

In the present study, dietary inclusion of HMC or rye in the diet of conventional chicks was associated with an increase in concentration of lactic acid and a decrease in pH in the ileal digesta. Both effects are associated with an increased microbial activity in the small intestine of chicks fed a diet containing HMC or rye. Others have shown that microbial activity increased in the ileum when birds were fed diets containing rye (Wagner and Thomas, 1978; Langhout et al., 1998[b]), wheat (Choct et al., 1996) or HMC (Wagner and Thomas, 1978; Langhout et al., 1998[ab]). Results of the present study show that digestibility of starch in the ileum was not influenced when HMC was included in the diet. This indicates that the increase in microbial activity could not be explained by an increase in fermentation of starch. Therefore, it is most likely that fermentation of the water-soluble NSP fraction is mainly responsible for the increase in microbial activity.

The effect of elevated microbial activity in the small intestine on nutrient utilization is still not well understood. An increase in microbial activity in the small intestinal tract indicates that more nutrients are fermented instead of enzymatically hydrolysed and digested. The end-products of microbial degradation of carbohydrates are lactic acid, formic acid and VFA. Pigs can utilize these end-products as an energy source, albeit with a lower efficiency than glucose, for example. In pigs, the losses are reported to vary between 33% (ARC, 1981) and 50% (Just et al., 1983; Van Es, 1987). For poultry no literature data are available. However, it may be assumed that these losses are of similar magnitude as in pigs. Moreover, bacteria are able to incorporate amino acids in microbial protein (Salter and Coates, 1974). This may explain the lower N utilization in conventional birds compared to germ-free birds as shown in the present study and by Furuse and Yokota (1985). In addition, many bacterial species are able to deconjugate bile acids (Hylemond, 1985). Therefore, an increase in microbial activity in the intestinal tract may increase bile acid deconjugation. This is supported by the results of Langhout et al. (1998[b]), who showed an increase in concentration of unconjugated bile acids in birds fed diets containing HMC or rye and wheat. Deconjugated bile acids may impair micelle formation and consequently reduce digestion of fat, in particularly of fats containing long-chain saturated fatty acids (Garrett and Young, 1975).

Table 7. *Effect of highly methylated citrus pectin (HMC) and rye on the concentrations of lactic acid, formic acid and volatile fatty acids (VFA) in the ileal digesta (in mg/kg) of conventional chicks at 22 d of age.*

	Dietary treatment			SEM
	maize	maize + 30 g HMC/kg	rye	(df=20)
Lactic acid	1.57^{a}	2.13^{b}	1.85^{ab}	0.145
Formic acid	0.16	0.15	0.17	0.027
Total VFA	0.33	0.42	0.38	0.121
Acetic acid	0.06	0.14	0.17	0.105
Propionic acid	0.11	0.12	0.11	0.020
Iso-butyric acid	0.08^{a}	0.02^{b}	0.04^{ab}	0.014
Butyric acid	0.01	0.05	0.02	0.017
Iso-valeric acid	0.05	0.06	0.03	0.021
Valeric acid	0.03	0.03	0.01	0.017

^{a,b} Mean values within a row with no common superscripts differ significantly ($P<0.05$).

The results of the present study show that addition of HMC to the diet of conventional chicks affects morphology of the villi in the ileum. The same was true when rye was included in the diet. These results agree with those obtained in a previous study (Langhout et al., 1998^{a}). This change in gut morphology may have reduced nutrient absorption. In germ-free birds, HMC or rye had little effect on the morphology of the villi. Gee et al. (1996) concluded that the effect of gelling agents on mucosal conditions is dependent on their fermenting ability. Such products as amines, ammonia and toxins, produced by the intestinal microflora, have been suggested to have deleterious effects on the gut wall. Therefore, it can be speculated that a change in metabolic activity of the intestinal microflora affects gut morphology.

Table 8. *Effect of highly methylated citrus pectin (HMC) and rye on classification and shape of the villi in the ileum of 22-day old conventional and germ-free chicks.*

	Dietary treatment			SEM
	maize	maize + 30 g HMC/kg	rye	
Conventional chicks				(df=15)
Classification of the villi (% of total)				
Zigzag	61^a	7^c	32^b	19.3
Shape of the villi (% of total)				
Tongue-shaped	23^a	54^b	40^b	18.5
Leaf-shaped	36	42	39	17.3
Ridge-shaped	35^a	1^c	17^b	16.9
Convoluted	5	4	4	4.4
Germ-free chicks				(df=8)
Classification of the villi (% of total)				
Zigzag	31	43	41	9.1
Shape of the villi (% of total)				
Tongue-shaped	33	32	38	6.5
Leaf-shaped	39	43	35	3.1
Ridge-shaped	24	24	25	5.8
Convoluted	4	1	5	1.8

^{a,b,c} Mean values within a row with no common superscripts differ significant ($P<0.05$).

Based on the results of the present study, it can be concluded that the gastrointestinal microflora mediates the magnitude of the anti-nutritive effects of water-soluble NSP in broiler chicks by affecting viscous properties and fermentation of water-soluble NSP in the gastrointestinal tract. The exact role of the microflora in chicks on the magnitude of the anti-nutritive effects of NSP needs to be further defined.

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References

Agricultural Research Council (1981) The nutrient requirement of pigs. Slough: Commonwealth Agricultural Bureaux.

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

Antoniou, T., Marquardt, R.R. & Cansfield, P.S. (1981) Isolation, partial characterization and antinutritional activity of a factor (pentosans) in rye grain. *Journal of Agricultural Food and Chemistry* 28: 1240-1247.

Association of Official Analytical Chemists (1984) *Official Methods of Analsis*, 14th edn (Washinton, DC, AOAC).

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens; effect of microbial status, diet gamma irradation and sodium taurocholate supplementation *British Poultry Science* 24: 191-203.

Choct, M. & Annison, G. (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M., Annison, G. & Trimble, R.P. (1992) Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition* 122: 2457-2465.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Coates, M.E., Cole, C.B., Fuller, R., Houghton, S.B. & Yokota, H. (1981) The gut microflora and the uptake of glucose from the small intestine of the chick. *British Poultry Science* 22: 289-294.

Dutch Bureau of Livestock Feedingstuffs (1994) Chemical composition, digestibility and energy value of feed ingredients. Bureau of Livestock Feedingstuffs, Lelystad, The Netherlands.

Edwards, C.A., Johnson, I.I. & Read, N.W. (1988) Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? *European Journal of Clinical Nutrition* 42: 307-312.

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

Furuse, M. & Yokota, H. (1985) Effect of the gut microflora on chick growth and utilization of protein and energy at different concentrations of dietary protein. *British Poultry Science* 26: 97-104.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Gee, J.M., Lee-Finglas, W., Wortley, G.W. & Johnson, J.T. (1996). Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. *Journal of Nutrition* 126: 373-379.

Hill, F.W. & Anderson, D.L. (1958) Comparison of metabolizable energy and productive energy determinations with growing chicks *Journal of Nutrition* 64: 587-603.

Hylemond, P.B. (1985) Metabolism of bile acids in intestinal microflora In: Sterols and bile acids: New Comprehensive Biochemistry (Danielsen, H. & Sjövall, J., eds.) Elsevier Science Publishers B.V. pp 331-343.

Just, A., Fernande, J.A. & Jorgensen, H. (1983) The net energy value of diets for growth in pigs in relation to the fermentative processes in the digestive tract and the site of absorption of the nutrients. *Livestock Production Science* 10: 171-186.

Langhout, D.J. & Schutte, J.B. (1996) Nutritional implications of pectins in chicks in relation to esterification and origin of pectins. *Poultry Science* 75: 1236-1242.

Langhout, D.J., Schutte, J.B., Van Leeuwen, P., Wiebenga, J. & Tamminga, S. (1998^a) Effect of dietary high- and low-methylated citrus pectin on activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. *British Poultry Science* (in press).

Langhout, D.J., Schutte, J.B., Tangerman, A., Verstraten, A.J.M.A., Van Schaik, A. & Beelen, G. (1998^b) Effect of dietary viscous polysaccharides on the ileal microflora and on bile acid deconjugation in broiler chicks. *British Poultry Science* (submitted).

National Research Council (1994) Nutrient requirements of poultry, 9th revised edn (Washington DC, National Academy of Sciences).

Norusis, M.J. (1992) SPSS/PC+ Base System User's Guide Version 5.0 (SPSS inc., Chicago, Illinois).

Salter, D.N. & Coates, M.E. (1974) The utilization of protein and excretion of uric acid in germ-free and conventional chicks. *British Journal of Nutrition* 31: 307-318.

Slump, P. (1969) Karakterisering van de voederwaarde van eiwitten in voedermiddelen door hun aminozuursamenstelling en de invloed van verhitting en loogbehandeling op de benutbaarheid van aminozuren. *PhD Thesis*, Free University Amsterdam, Amsterdam, The Netherlands.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical methods. 7th ed. (The Iowa State University Press. Ames, 1A).

Van Es, A.J.H. (1974) Energy utilization of low digestibility carbohydrates. In: *Low digestibility carbohydrates* (Leegwater, D.C., Feron, V.J. & Hermus, R.J.J., eds.). Wageningen: Pudoc Press, pp. 121-127.

Van der Klis, J.D., Verstegen, M.W.A. & Van Voorst, A. (1993) Effect of a soluble polysaccharide (Carboxy Methyl Cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. *British Poultry Science* 34: 377-389.

Wagner, D.D. & Thomas O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

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

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1981) The viscosity interaction of barley ß-glucan with Trichoderma viride cellulase in the chick intestine. *Poultry Science* 62: 853-862.

General discussion

General discussion

The investigations described in this thesis are mainly focused on the influence of chemical and physical properties of water-soluble non-starch polysaccharides (NSP) on ileal microbial activity and composition in relation to nutrient digestibility and performance in broiler chicks. In this Chapter several aspects of the results are compared with literature data and discussed in relation to some practical implications.

Viscous water-soluble NSP

In broiler chick diets, the carbohydrate fraction is the primary source affecting microbial activity in the intestinal tract. The effect of carbohydrates on the microflora depends on the type of carbohydrate (Chapter 1). In general, the results of the literature review in Chapter 1 indicate that water-soluble carbohydrates increase microbial activity in the intestinal tract, whereas insoluble carbohydrates may decrease microbial activity. It is generally accepted that the water-soluble NSP present in barley, rye and wheat, depress performance and reduce nutrient utilization in chicks. These anti-nutritive effects are related to the presence of the viscous ß-glucans in barley (White *et al.*, 1981; Fincher and Stone, 1986), and to the presence of the viscous arabinoxylans (pentosans) in rye (Antoniou *et al.*, 1981; Ward and Marquardt, 1983) and in wheat (Choct and Annison 1990; 1992). It has generally been suggested that the viscous properties of the water-soluble NSP are responsible for their anti-nutritive properties (Ward and Marquardt, 1983; Choct and Annison, 1992[a]; Bedford and Classen, 1993).

It is well documented that addition of ß-glucanase- or xylanase-containing enzyme preparations to barley-based (Hesselman *et al.*, 1981; Hesselman and Åman, 1986), rye-based (GrootWassink *et al.*, 1989; Bedford and Classen, 1993) and wheat-based diets (Annison, 1992; Schutte *et al.*, 1993) can eliminate, at least in part, the anti-nutritive effects of ß-glucans and pentosans in chicks. In Chapter 2 it was shown that the effect on performance of an endo-xylanase supplementation to a wheat- and rye-based diet was dependent on the type of dietary fat source. The improvement in broiler performance after supplementation of the endo-xylanase to a wheat/rye-based diet was more pronounced when blended animal fat instead of soya oil was included in the diet. The type of fat, however, had no effect on digesta viscosity (Chapter 5). These results suggest that factors other than digesta viscosity are also responsible for the anti-nutritive properties of water-soluble NSP. With regard to the latter, several investigators have suggested that the negative effect of water-soluble NSP is mediated by the intestinal microflora (Wagner and Thomas, 1978; Campbell *et al.*, 1983; Choct and Annison, 1992^{b}; Choct *et al.*, 1992; 1996).

Physical and chemical properties of water-soluble NSP

Previous studies on the anti-nutritive effects of water-soluble NSP were mainly focused on their viscous properties (Van der Klis, 1993; Smits, 1996). In these studies the unfermentable water-soluble carboxymethylcellulose (CMC) was used as a model product. Water-soluble NSP present in feedstuffs, however, not only have viscous properties, but are generally also fermented (Roberfroid, 1993) in the intestinal tract of chicks. Therefore, dietary water-soluble NSP *per se* may increase microbial activity in the intestinal tract.

Sakata (1987) observed a change in the morphology of the gastrointestinal wall with increasing bacterial activity in the intestinal tract. A change in the mucosa of the small intestine was also observed when including barley or rye in the diet (Viveros *et al.*, 1994; Silva and Smithard, 1996). Smits (1996), however, showed no effect on the morphology of the small intestine when CMC was included in broiler chick diets. In addition, Gee *et al.* (1996) showed that addition of CMC to rat diets hardly affected the morphology of the intestinal wall, whereas addition of citrus pectin to the diet markedly changed the morphology of the gut wall. Citrus pectin has viscous properties and can also be fermented by the intestinal microflora. Therefore, the fermentability of water-soluble NSP might be an important factor with regard to their physiological effects. Because of this, water-soluble, viscous, fermentable citrus pectin was chosen as a model product in the studies (Chapters 3, 4, 5, and 6).

The anti-nutritive properties of citrus pectin depend on their structure (Chapter 3). Addition of highly methylated citrus pectin (HMC) to the diet depressed broiler performance, whereas the inclusion of low-methylated citrus pectin (LMC) in the diet had little or no effect on performance. This difference in anti-nutritive effects between both pectin products could not be explained by their viscous properties. HMC easily forms a gel with sugars and acid, whereas LMC forms a gel with cations such as calcium, regardless the pH value (May, 1990). HMC seems to have similar effects on the viscosity of the diet as LMC (Chapter 2), indicating that both sugars and cations were present in the diet. However, the effect of both pectin products on ileal viscosity was different (Chapters 4 and 5); the increase in digesta viscosity was more pronounced in birds fed the HMC diet than in birds fed the LMC diet. The increase in water/feed ratio when HMC or LMC was included in the diet, was similar for both pectin products (Chapter 5), or slightly higher in birds fed the HMC diet (Chapters 3 and 4). This indicates that the difference in digesta viscosity between both pectin products could not be explained by a difference in water consumption.

In germ-free chicks the increase in digesta viscosity in birds fed the HMC diet was lower than in conventional birds fed the same diet (Chapter 6). This difference in digesta viscosity data between germ-free and conventional birds indicates that the intestinal microflora mediates the physical properties of water-soluble NSP. This difference in digesta viscosity between conventional and germ-free birds fed either a HMC or a rye-based diet was already present in the proximal part of the small intestine (Chapter 6). It had been reported that birds fed a rye-based diet have higher levels of cholyltaurine hydrolase in the crop than those fed a corn-based diet (Feigner and Dashkevicz, 1988). Cholyltaurine hydrolase is produced by bacteria. Thus, inclusion of water-soluble NSP in broiler diets might increase microbial activity in the crop and the intestinal tract, resulting in (partial) modification of the NSP fraction and in an increase in digesta viscosity.

The results described in Chapter 6 also show that the intestinal microflora increases the viscous properties of HMC. It might be that the microflora breaks down HMC into shorter galacturonic units of higher viscous properties. Indications that HMC is at least partly fermented by the intestinal microflora were obtained in the studies described in Chapters 4 and 6, showing a higher digestibility of the non-starch carbohydrate fraction when including HMC in the diet of conventional chicks, than with HMC in the diet of germ-free chicks (Table 1). The results listed in this Table show that dietary addition of LMC hardly affects the digestibility of the non-starch carbohydrate fraction as compared to the control (maize) diet. This indicates that HMC is, at least in part, fermented by the intestinal microflora, whereas LMC is hardly fermented. This notion is supported by the results of Chapters 4 and 5, in which an increase in microbial activity was found in birds fed the HMC diet, whereas LMC hardly affected microbial activity. The difference between HMC and LMC is related to their degree in esterification of carboxyl groups. Therefore, the degree of esterification of pectins might influence the ability of the intestinal microflora to ferment a pectin product.

In practice, differences in nutritional quality are found among wheat batches. These differences in quality of wheat batches could only partly be explained from differences in content of water-soluble pentosans or *in vitro* viscosity (Annison, 1991). Results of our studies indicate that differences in quality of feedstuffs containing water-soluble

NSP may also be related to differences in structure and composition of water-soluble NSP fractions. Several factors, such as the growing conditions of wheat and climate during harvesting, influence the structure of pentosans. Differences in the structure of pentosans may not only affect their physical properties (Voragen *et al.*, 1994), but also their fermentability.

Table 1. *Apparent faecal digestibility of non-starch carbohydrates calculated on the basis of the apparent faecal digestibility values reported in Chapters 4 and 6.*

Chapter	Diet	Total non-starch carbohydrates (g/kg of diet)	Digestible non-starch carbohydrates[1] (g/kg of diet)
4			
	maize	211	- 2
	maize + 30 g HMC/kg	211	+ 14
	maize + 30 g LMC/kg	211	- 9
6			
	Conventional chicks		
	maize	179	+ 10
	maize + 30 g HMC/kg	209	+ 22
	Germ-free chicks		
	maize	179	+ 9
	maize + 30 g HMC/kg	209	+ 5

[1] Calculated as digestible non-starch carbohydrates = digestible organic matter - (digestible crude fat + digestible crude protein + digestible starch). The mean apparent faecal digestibility coefficient of the amino acids was used for crude protein.

The gut microflora and nutrient digestion

The addition of HMC to broiler diets depressed weight gain and feed utilization (Chapters 3, 4, 5 and 6). The same is true when birds were fed a diet containing rye (Chapter 6) or rye and wheat (Chapter 5). The negative effect of HMC on broiler performance was, at least in part, due to a reduction in nutrient digestibility (Chapters

4 and 6). However, when germ-free chicks were fed a diet containing HMC or rye, feed utilization and nutrient digestibility were hardly affected (Chapter 6). These results indicate that the depression in nutrient digestibility in conventional chicks by including water-soluble NSP in their diet is mainly attributable to the intestinal microflora.

The negative effects of HMC, rye and wheat are associated with an increase in microbial activity in the ileum (Chapters 4 and 5). It is suggested that the increase in digesta viscosity caused by these products lowers the rate of hydrolysis of nutrients in the proximal part of the small intestine. As a consequence, more undigested nutrients enter the ileum, and microbial growth will be increased. Results of the study described in Chapter 6 indicated that digestibility of starch in the ileum was not influenced when HMC was included in the diet. This indicates that the increase in microbial activity could not be explained by an increase in fermentation of starch. Therefore, it is most likely that fermentation of the water-soluble NSP fraction is mainly responsible for the increase in microbial activity.

The mechanism by which the microflora influences nutrient digestion is still not fully understood. As discussed in Chapter 6, the microflora may affect several physiological mechanisms which impair nutrient absorption, nutrient utilization and broiler performance. Figure 1 summarizes the mechanism by which microbial activity, after ingestion of water-soluble NSP, may depress these parameters. An increased fermentation of nutrients by the microflora, instead of enzymatic digestion, is associated with energy losses. In addition, amino acids are fermented or incorporated into the bacterial mass, as shown by Salter and Coates (1974). This may, at least in part, explain the lower nitrogen utilization in birds fed the HMC diet (Chapters 4 and 6).

Addition of HMC to the diet particularly reduced digestibility of fat (Chapters 4 and 6). It is well documented that intestinal bacteria affect fat digestibility (Chapter 1). Most of the bacteria present in the intestinal tract are able to transform bile acids trough deconjugation, dehydration and dehydrogenation (Hylemond, 1984). Of these different transformation forms of bile acids, the most common reaction is deconjugation. This is supported by the results of Chapter 5, in which an increase in the concentration of unconjugated bile acids was found in birds fed diets containing HMC, LMC or rye and wheat. Deconjugated bile acids may impair micelle formation. As a consequence, absorption of fatty acids is reduced (Coates *et al.*, 1981). In this respect, the digestion of long chain and saturated fatty acids would be more sensitive to any inadequacy than short chain and long chain unsaturated fatty acids (Krogdahl, 1985). Moreover, Garrett and Young (1975) showed that short chain or unsaturated fatty acids are more easily absorbed without micelles being formed. These findings are supported by the results

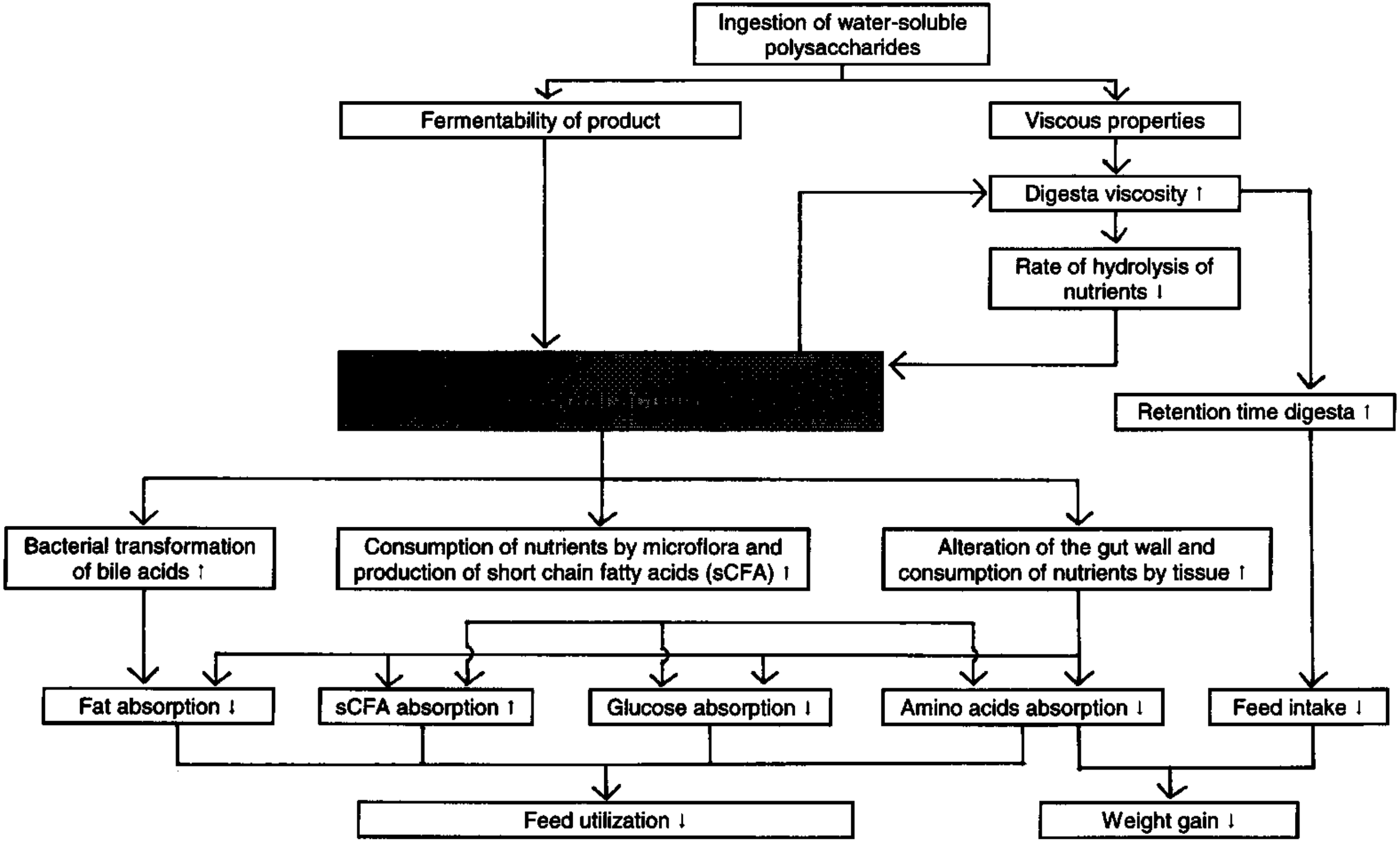

Figure 1. *The mechanism by which water-soluble NSP may depress nutrient digestibility and broiler chick performance.*

of our study described in Chapter 5. In this Chapter a greater depression in broiler performance was found when blended animal fat instead of soya oil was included in the diet.

Supplementation of HMC and rye added to the diet alters the morphology of the gut wall in conventional chicks (Chapters 4 and 6), whereas hardly any effect on gut morphology was observed in germ-free chicks (Chapter 6). These results indicate that the effect of the water-soluble NSP on the gut wall is mediated by the intestinal microflora. Angkanaporn *et al.* (1994) showed an increase in endogenous losses of amino acids when wheat pentosans were added to broiler diets. Addition of HMC to the diet increased the number of goblet cells per 100 villus cells (Chapter 4). Goblet cells are responsible for the secretion of mucin of the intestinal epithelium (Schneeman, 1982). Thus, a higher density in goblet cells may result in an increase in the secretion of mucin. Changes in mucin excretion may influence nutrient requirement for the gut. An increase in the number of goblet cells may also indicate that cell proliferation is increased. An increase in cell proliferation will reduce the age and maturity of the goblet cells. As a consequence, the absorption of nutrients might be reduced (Hampson, 1986).

The mechanism by which the microflora affects the gut wall is not clear. Gee *et al.* (1996) concluded that the effect of gelling agents on the gut mucosa was dependent on their fermentability. It has been suggested that products such as amines, ammonia and toxins, produced by the intestinal flora, have deleterious effects on the gut wall. Therefore, changes in metabolic activity of the intestinal microflora due to the presence of water-soluble NSP in the diet may have an effect on the morphology and histology of the gut wall.

In practice, broiler diets are normally supplemented with a growth-promoting antibiotic. A change in the composition, activity and/or metabolism of the gastrointestinal microflora is assumed to be the primary mode of action of these growth-promoting antibiotics (Mackinnon, 1985). There is, however, an increasing public reluctance towards the use of these growth-promoting antibiotics in animal diets due to an increasing concern regarding the possible emergence of antibiotic resistant strains of species pathogenic to man. Therefore, new types of broiler chick diets have to be developed including alternative feed additives which are more 'natural' in concept. Results of this thesis demonstrate that dietary factors can easily change the microflora in the intestinal tract, and as a result performance of broiler chicks can be affected. Therefore, these new broiler chick diets should aim at controlling microbial activity in order to optimize nutrient digestibility and performance of broiler chicks.

The microflora and the health status of the bird

There is ample evidence that the indigenous microflora influences the health status of the host (Chapter 1). Birds fed diets based on barley, rye or wheat are reported to have more health problems particularly with regard to Necrotic enteritis than birds fed on maize-based diets (Kaldhusdal and Skjerve, 1996; Elwinger *et al.*, 1992). Necrotic enteritis is caused by toxins produced by *Clostridium perfringens* type A. Inclusion of barley in a wheat-based diet increased the number of *Clostridia* in the small intestine (Kaldhusdal and Hofshagen, 1992). The addition of HMC to the diet also affected the composition of the gut ecosystem (Chapters 4 and 5). Particularly the count of *Enterococci* and *Escherichia coli* and, to a lower extent, also of *Clostridium* increased when birds were fed HMC. In birds fed the rye/wheat based diet the number of *E. coli* increased (Chapter 5). In our studies, birds were kept in battery cages under favourable environmental conditions. In practice, environmental conditions are less favourable. Therefore, it can be speculated that the health status of birds fed diets containing water-soluble NSP is negatively affected due to a disturbed balance in the gut ecosystem.

There is only limited information about the mechanism by which growth of bacteria is affected. It is questionable whether this effect can be completely explained by competition for receptor places on the villus surface, since most of the bacteria colonize in the lumen (Rosebury, 1962). It is most likely that changes in environmental conditions in the intestinal tract influence growth of bacteria. In birds fed diets containing water-soluble NSP more undigested nutrients (mainly starch, amino acids and NSP) become available for fermentation. This increase in fermentation may play an essential role in the change in the microbial composition. It might be that several bacteria like *Enterococci* and *E. coli* dominate other bacteria in the competition for fermentation of water-soluble NSP.

Addition of HMC or rye to the diet decreased ileal pH (Chapters 5 and 6) and increased the concentration of lactic acid (Chapter 6). It is generally accepted that growth of Gram-negative microorganisms in the intestinal tract is inhibited at low pH and/or at high levels of lactic acid. The reduction in *Salmonella* colonization associated with products like lactose and oligosaccharides linked to galactose, fructose or mannose have been suggested to be due to a reduction in pH or an increase in lactic acid concentration (Hidaka *et al.*, 1986; Corrier *et al.*, 1991[a,b]; Hinton *et al.*, 1991; Baily *et al.*, 1991; Waldroup *et al.*, 1993). However, results of the studies described in Chapters 4 and 5 show that a reduction in pH and an increase in the level of lactic acid was accompanied with an increase in the number of *E. coli* . Therefore, it can be argued whether lowering the pH or increasing the concentration of lactic acid in the distal part of the intestinal tract is the mechanism by which colonization of Gram-negative bacteria such as *Salmonella* is reduced.

Conclusions

On basis of the results of the studies described in this thesis with broiler chicks fed diets containing water-soluble NSP the following conclusions can be drawn.

- Inclusion of water-soluble NSP in broiler diets results in an increase in the microbial activity and a change in the composition of the microflora in the small intestine. This increase in microbial activity and change in microbial composition mediates the magnitude of the anti-nutritive effects of these NSP fractions on nutrient digestibility and performance of broiler chicks by affecting viscous properties and fermentation of water-soluble NSP in the gastrointestinal tract.
- The increase in microbial activity after consumption of water-soluble NSP stimulates deconjugation of bile acids in the ileum of broiler chicks. This increase in bile acid deconjugation may partly explain the reduction in fat digestibility when water-soluble NSP are included in the diet.
- The increase in microbial activity and the change in microbial composition in the ileum of birds fed diets containing water-soluble NSP alter gut morphology. These changes in the gut wall may affect nutrient absorption.
- The increase in digesta viscosity is not the only explanatory factor for the anti-nutritive effects of water-soluble NSP on broiler performance and nutrient digestibility.

References

Angkanaporn, K., Choct, M., Bryden, W.L. & Annison, E.F. (1994) Effects of wheat pentosans on endogenous amino acid losses in chickens. *Journal of the Science of Food and Agriculture* 66: 399-404.

Annison, G. (1991) Relationship between the levels of non-starch polysaccharides and the apparent metabolisable energy of wheats assayed in broiler chickens. *Journal of Agriculture and Food Chemistry* 39: 1252-1256.

Annison, G. (1992) Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler chickens. *Animal Feed Science and Technology* 38: 105-121.

Antoniou, T., Marquardt, R.R. & Cansfield, E. (1981) Isolation, partial characterization and anti-nutritional activity of a factor (pentosans) in rye grain. *Journal of Agriculture and Food Chemistry* 29: 1240-1247.

Bailey, J.S., Blankenship, L.C. & Cox, N.A. (1991) Effect of fructooligosaccharides on *Salmonella* contamination of the chicken intestine. *Poultry Science* 70: 2433-2438.

Bedford, M.R. & Classen, H.L. (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 Science* 72: 137-143.

Campbell, G.L., Campbell, L.D. & Classen, H.L. (1983) Utilisation of rye by chickens; effect of microbial status, diet gamma irradiation and sodium taurocholate supplementation. *British Poultry Science* 24: 191-203.

Choct, M. & Annison, G. (1990) Antinutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811-821.

Choct, M. & Annison, G. (1992^a) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* 67: 123-132.

Choct, M. & Annison, G. (1992^b) Anti-nutritive effect of wheat pentosans in broiler-chickens: Role of viscosity and gut microflora. *British Poultry Science* 33: 821-834.

Choct, M., Annison, G. & Trimble, R.P. (1992) Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition* 122: 2457-2465.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. & Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609-621.

Coates, M.E., Cole, C.B., Fuller, R., Houghton, S.B. & Yokota, H. (1981) The gut microflora and the uptake of glucose from the small intestine of the chick. *British Poultry Science* 22: 289-294.

Corrier, D.E., Hargis, B., Hinton, A. Jr., Lindsey, D., Caldwell, D., Manning, J. & DeLoach, J.R. (1991^a) Effect of anaerobic cecal microflora and dietary lactose on colonization resistance of layer chicks to invasive *Salmonella enteritidis*. *Avian Diseases* 35: 337-343.

Corrier, D.E., Hinton, A. Jr., Kubena, L.F., Ziprin, R.L. & DeLoach, J.R. (1991^b) Decreased *Salmonella* colonization in turkey poults inoculated with anaerobic cecal microflora and provided dietary lactose. *Poultry Science* 70: 1345-1350.

Elwinger, K., Engström, Berndtson, E., Fossum, O. & Teglöf, B. (1992) The effects of Narasin on *Clostridium perfringens* in caeca and the occurrence of Necrotic enteritis in broiler chicks. *Proceedings of the XIX World's Poultry Congress*, Amsterdam, the Netherlands pp. 580-584.

Feighner, S.D. & Dashkevicz, M.P. (1988) Effect of dietary carbohydrates on bacterial cholyltauryl hydrolase in poultry intestinal homogenates. *Applied Environmental Microbiology* 54: 337-342.

Fincher, G.B. & Stone, B.A. (1986) Cell walls and their components in ceral grain technology. In: *Advances in Cereal Science and Technology, Volume 8* (Pomeranz, Y., ed.), Minnesota, AACC, pp. 207-295.

Garrett, R.L. & Young, R.J. (1975) Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. *Journal of Nutrition* 105: 827-838.

Gee, J.M., Lee-Finglas, W., Wortley, G.W. & Johnson, J.T. (1996). Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. *Journal of Nutrition* 126: 373-379.

GrootWassink, J.W.D., Campbell, G.L. & Classen, H.L. (1989) Fractionation of crude pentosanase (arabinoxylanase) for improvement of the nutritional value of rye diets for broiler chickens. *Journal of Food Agriculture* 46: 289-300.

Hampson, D.J. (1986) Alteration in piglet small intestinal structure at weaning. *Research in Veterinary Science* 40: 3.

Hesselman, K. & Aman, P. (1986) The effect of ß-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low or high viscosity. *Animal Feed Science and Technology* 15: 83-93.

Hesselman, K., Elwinger, K., Nilsson, M. & Thomke, S. (1981) The effects of ß-glucanase supplementation, stage of ripeness and storage treatment of barley in diets fed to broiler chickens. *Poultry Science* 60: 2664-2671.

Hidaka, H., Edida, T., Takazawa, T., Tokunga, T. & Tashiro, Y. (1986) Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* 5: 37-50.

Hinton, Jr., A., Corrier, D.E., Ziprin, R.L., Spates, G.E. & DeLoach, J.R. (1991) Comparison of the efficacy of cultures of cecal anaerobes as inocula with or without dietary lactose. *Poultry Science* 70: 67-73.

Hylemond, P.B. (1985) Metabolism of bile acids in intestinal microflora In: *Sterols and bile acids: New Comprehensive Biochemistry* (Danielsen, H. & Sjövall, J., eds.) Elsevier Science Publishers B.V., the Netherlands, pp. 331-343.

Kaldhusdal, M. & Skjerve, E. (1996) Association between ceral contents in the diet and incidence of Necrotic enteritis in broiler chickens in Norway. *Preventive Veterinary Medicine* 28: 1-16.

Kaldhusdhal, M. & Hofshagen, M. (1992) Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. *Poultry Science* 71: 1145-1153.

Krogdahl, A. (1985) Digestion and absorption of lipids in poultry. *Journal of Nutrition* 115: 675-685.

MacKinnon, J.D. (1985) The efficacy, benefits and general use of antimicrobial growth promoters in animal nutrition. In: *Proceedings of the Symposium "Criteria and Methods for the Microbiological Evaluation of Growth Promoters in Animal Feeds"* (Helmuth, R. & Bulling, E., eds.) Berlin, Germany, pp. 5-27.

May, M.B. (1990) Industrial pectin: sources, production and applications. *Carbohydrate Polymers* 12: 79-99.

Roberfroid, M. (1993) Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition* 33: 103-148.

Rosebury, T. (1962) Micro-organisms indigenous to man. McGraw-Hill Book Co. Inc. New York, p. 435.

Salter, D.N. & Coates, M.E. (1974) The utilization of protein and excretion of uric acid in germ-free and conventional chicks. *British Journal of Nutrition* 31: 307-318.

Sakata, T. (1987) Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal throphic factors. *British Journal of Nutrition* 58: 95-103.

Schutte, J.B., Geerse, C. & De Jong, J. (1993^a) Effect of ß-glucanase supplementation to a barley-based diet on broiler chick performance. *Proceedings World Poultry Science Association*, Jelenia Gora, Poland. pp. 490-492.

Schutte, J.B., Geerse, C. & De Jong, J. (1993^b) Effect of enzyme supplementation to wheat-based diets on broiler chicken performance. *Proceedings Enzyme Congress*, Switzerland pp. 133-136.

Silva, S.S. & Smithard, R.R. (1996) Extrogenous enzymes in broiler diets: crypt cell proliferation, digesta viscosity, short chain fatty acids and xylanase in the jejunum. *British Poultry Science* 37 (Suppl.): S77-S79.

Smits, C.H.M. (1996) Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. PhD thesis, Agricultural University Wageningen, the Netherlands.

Van der Klis, J.D. (1993) Physico-chemical chyme conditions mineral absorption in broilers. PhD thesis, Agricultural University Wageningen, the Netherlands.

Viveros, A., Brenes, A., Pizarro, M. & Castano, M. (1994) Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Animal Feed Science and Technology* 48: 237-251.

Voragen, A.G.J., Beldman, G., Gruppen, H. Schols, H.A., Verbruggen, M.A. & Vinken, J.-P. (1994) Enzymatic modification of algal and plant polysaccharides. *Carbohydrates in Europe* 10: 21-26.

Wagner, D.D. & Thomas, O.P. (1978) Influence of diets containing rye or pectin on the intestinal flora of chicks. *Poultry Science* 57: 971-975.

Waldroup, A.L., Skinner, J.T., Hierholzer, R.E. & Waldroup, P.W. (1993) An evaluation of fructooligosaccharide in diets for broiler chickens and effects on salmonellae contamination of carcasses. *Poultry Science* 72: 643-650.

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

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1981) The viscosity interaction of barley ß-glucan with Trichoderma viride cellulase in the chick intestine. *Poultry Science* 62: 853-862.

Summary

Summary

To-days broiler chicks have poor immunocompetence, resulting in a high prevalence of health problems resulting in metabolic disorders. These health problems are connected with a disturbance in the microbial ecosystem in the intestinal tract. In broiler diets, the carbohydrate fraction is the primary source affecting intestinal microbial activity. The effect of carbohydrates on intestinal microbial activity depends mainly on the source of the carbohydrate fraction. It is well recognized that water-soluble non-starch polysaccharides (NSP) depress the digestibility of nutrients, in particular of fat, in broiler chicks. The negative effects of water-soluble NSP have been associated with an increase in digesta viscosity. Water-soluble NSP do not only have a viscous nature but can also be fermented by the gut microflora. Therefore, it is likely that water-soluble NSP affect microbial activity in the intestinal tract.

The objective of the studies presented in this thesis was to determine the effect of the intestinal microflora as affected by chemical and physical properties of water-soluble NSP on broiler performance and nutrient digestibility. More specifically, the mechanisms by which the intestinal microflora affect nutrient and energy digestibility in broiler chicks were investigated using citrus pectin as a model substance. Citrus pectin is a water-soluble, fermentable fibre of high viscosity.

A literature review was conducted to investigate the effects of carbohydrates on microbial activity in the intestinal tract and subsequent effects on nutrient digestion in broiler diets (**Chapter 1**). Literature data indicate that water-soluble NSP increase microbial activity in the intestinal tract of broiler chicks. It was suggested that the negative effects of water-soluble NSP on broiler performance are mediated by the intestinal microflora. The mechanism by which the microflora affects nutrient digestion has not been elucidated. An increase in microbial activity in the small intestine might reduce hydrolysis and absorption of nutrients. It was hypothesized that the intestinal microflora may influence fat digestion through transformation of bile acids. In addition, protein and starch digestion may be impaired through increased competition between the host and the microflora. In addition, absorption of nutrients may be reduced through changes in morphology of the intestinal wall.

The purpose of the study described in **Chapter 2** was to investigate whether the effects of a dietary endo-xylanase enzyme preparation in a wheat- and rye-based diet on performance and nutrient digestibility were mediated by the source of fat in the diet. The fat sources examined were soya oil and blended animal fat. Addition of endo-xylanase to the diet with soya oil had little effect on performance and nutrient

digestibility. However, when the endo-xylanase preparation was added to the blended animal fat diet, weight gain, feed utilization and digestibilities of nutrients, nitrogen retention and metabolizable energy content were improved. The improvement in fat digestibility was most pronounced. From the results of this study, it was concluded that the effects on chick performance and nutrient digestibility by endo-xylanase supplementation to a wheat- and rye-based diet are largely influenced by the source of fat in the diet. Furthermore, the results of this study indicate that factors other than viscosity are also responsible for the anti-nutritive properties of water-soluble NSP.

Chapter 3 describes an experiment conducted to examine the effect of feeding highly methylated citrus pectin (HMC), low-methylated citrus pectin (LMC) and high-methylated sugar beet pectin (HMS), at dietary levels of 15 and 30 g/kg in a maize soya-based diet, on performance and physiological characteristics in broiler chicks. The inclusion of LMC or HMS in the diet had little effect on the performance of chicks; however, when HMC was added to the diet growth and feed utilization were adversely affected. The effect of HMC on growth and feed utilization was dose dependent. Water intake and water/feed ratio were increased after inclusion of all three pectin products in the diet, the effect being most pronounced for the HMC-containing diets. The viscosity of the diet was increased by incorporating HMC or LMC in the diet, whereas HMS had no effect. Water-holding capacity of the diets and the excreta were increased by including one of the three pectin products in the diet. Addition of HMC to the diet decreased the concentration of volatile fatty acids (VFA) in caecal digesta, whereas LMC had no effect on the concentration of VFA. In contrast, inclusion of HMS in the diet increased the concentration of VFA in caecal digesta. Based on the results of this study, it was concluded that the effect of dietary pectin on chick performance is dependent on the degree of carboxyl groups esterified, the origin of the pectin product and the dietary dose level.

In **Chapter 4** results are reported of a study for determining the effects of dietary HMC and LMC on performance, nutrient digestibility, morphology of the villi of the small intestine and ileal microbial activity in broiler chicks. Both pectin products were tested at a dietary level of 30 g/kg in a maize soya-based diet. Inclusion of HMC in the diet depressed performance and decreased digestibility of most nutrients in particular that of fat. With a dietary addition of LMC performance of chicks was not affected, only fat digestion was decreased. Inclusion of HMC and LMC in the diet increased digesta viscosity. Microbial activity in the ileum, particularly that of *Enterococci*, *Bacteroidaceae*, *Clostridium* spp. and *Escherichia coli*, was increased by dietary addition of HMC. In addition, the morphology of the ileal wall was markedly affected in birds fed the HMC diet and the number of goblet cells per 100 villus cells as well as sucrase isomaltase activity were increased. Inclusion of LMC in the diet did not greatly affect microbial activity as only the number of *Clostridium* spp. was increased. Also morphology of the intestinal wall was hardly affected by LMC, whereas the number of goblet cells per 100 villi cells was significantly increased. Based on the results of this study it was concluded that water-soluble pectins change the ecosystem of the ileum and the morphology and histology of the ileal wall. The magnitude of these changes depends on the degree of methylation of the pectins.

Chapter 5 describes a study investigating the effects of HMC, LMC, wheat and rye on activity and composition of the microflora and degree of bile acid deconjugation in the small intestine in relation to the fat source in broiler chicks. Soya oil and blended animal fat were used as fat sources. Inclusion of HMC, LMC or rye/wheat in a maize soya-based diet significantly depressed performance of chicks, the effect being most pronounced when the diet contained blended animal fat compared to soya oil. Addition of HMC, LMC or rye/wheat to the diet reduced significantly the pH of ileal digesta, whereas the concentration of VFA in ileal digesta was increased only in birds fed the rye/wheat diet. Inclusion of HMC in the diet increased significantly the total count of aerobic and anaerobic bacteria including *Bifidobacteria*, *Enterococci* and *E. coli*. When birds were fed the rye/wheat-based diet only *E.coli* counts were raised. Inclusion of LMC in the diet little affected the microbial population or composition. The concentration of conjugated bile acids was significantly reduced when HMC, LMC or rye/wheat was included in the diet, whereas the corresponding level of unconjugated bile acids was increased. It was suggested that, in addition to digesta viscosity, an increase in microbial activity in the small intestine is at least partly responsible for the anti-nutritive properties of water-soluble NSP.

The purpose of the study described in **Chapter 6** was to obtain more information on the role of the intestinal microflora in the anti-nutritive properties of water-soluble NSP by using conventional and germ-free broiler chicks. The water-soluble NSP containing products involved in the study were HMC and rye. Feeding the HMC or the rye diet to conventional chicks depressed weight gain and feed utilization, whereas in germ-free chicks only weight gain was reduced significantly when HMC was included in the diet. Feeding the HMC diet to conventional chicks reduced digestibility of energy and starch at the end of the ileum. Faecal digestibility of nutrients, nitrogen retention and metabolizable energy were reduced when conventional chicks were fed the HMC diet. Feeding the HMC diet to germ-free chicks had little effect on faecal digestibility of nutrients and nitrogen retention, whereas metabolizable energy of the diet was increased. Feeding the HMC or the rye diet to conventional and germ-free chicks increased significantly digesta viscosity in the small intestine. This increase in digesta viscosity was more pronounced in conventional than in germ-free chicks. Feeding the HMC or the rye diet to conventional chicks markedly affected the morphology of the villi in the small intestine, whereas in germ-free chicks little effect was observed. Based on the results of this study it was concluded that the gastrointestinal microflora mediates the magnitude of the anti-nutritive effects of water-soluble NSP in broiler chicks by affecting viscous properties and fermentation of water-soluble NSP in the gastrointestinal tract.

In the **General discussion**, the results of these experiments were combined and some practical implications were discussed. The most important conclusions from the studies described in this thesis can be summarized as follows:

- Inclusion of water-soluble NSP in broiler diets results in an increase in the microbial activity and a change in the composition of the microflora in the small intestine. This increase in microbial activity and change in microbial composition mediates the magnitude of the anti-nutritive effects of these NSP fractions on nutrient digestibility and performance of broiler chicks by affecting viscous properties and fermentation of water-soluble NSP in the gastrointestinal tract.
- The increase in microbial activity after consumption of water-soluble NSP stimulates deconjugation of bile acids in the ileum of broiler chicks. This increase in bile acid deconjugation may partly explain the reduction in fat digestibility when water-soluble NSP are included in the diet.
- The increase in microbial activity and the change in microbial composition in the ileum of birds fed diets containing water-soluble NSP alter gut morphology. These changes in the gut wall may affect nutrient absorption.
- The increase in digesta viscosity is not the only explanatory factor for the anti-nutritive effects of water-soluble NSP on broiler performance and nutrient digestibility.

Samenvatting

Samenvatting

De pluimveeindustrie heeft in toenemende mate te kampen met gezondheidsproblemen bij vleeskuikens. Deze problemen worden veelal in verband gebracht met een verstoring van het ecosysteem in het maagdarmkanaal. Daarbij wordt verondersteld dat de huidige vleeskuikenvoeders zodanig zijn samengesteld dat hierdoor de ontwikkeling van een evenwichtig ecosysteem wordt belemmerd. Koolhydraten vormen in rantsoenen voor vleeskuikens de belangrijkste bron voor fermentatie en zijn daardoor de belangrijkste voedingsfactoren die de microflora in het darmkanaal beïnvloeden. Het effect van koolhydraten op de microflora hangt sterk af van de aard van de koolhydraat fractie. Het is bekend dat wateroplosbare niet-zetmeel polysacchariden (NSP) de vertering van nutriënten, met name die van vet, verlagen. Dit negatieve effect van wateroplosbare NSP wordt veelal in verband gebracht met een verhoging van de viscositeit in de dunne darm. Wateroplosbare NSP zijn niet alleen visceus van aard, maar kunnen ook worden gefermenteerd door de microflora in het maagdarmkanaal van het kuiken. Het is daarom aannemelijk dat water-oplosbare NSP van invloed zijn op de microbiële activiteit in het darmkanaal.

De doelstelling van dit proefschrift was het bestuderen van de invloed van water-oplosbare NSP op de activiteit en samenstelling van de microflora in het darmkanaal in relatie tot de vertering van nutriënten en de produktieresultaten van vleeskuikens. Citrus pectine werd gebruikt als model stof om het effect van de microflora op de verteerbaarheid van nutriënten en de produktieresultaten te onderzoeken. Citrus pectine is een wateroplosbare, fermenteerbare NSP fractie met een hoge viscositeit.

Op basis van een uitgevoerde literatuurstudie werd geconcludeerd dat wateroplosbare NSP de microbiële activiteit in het darmkanaal van vleeskuikens verhogen (**Hoofdstuk 1**). Er wordt daarbij verondersteld dat het negatieve effect van wateroplosbare NSP op de produktieresultaten wordt gemedieerd door een verhoging van de microbiële activiteit in het maagdarmkanaal. Middels welk mechanisme de microflora de vertering van nutriënten beïnvloedt is niet volledig bekend. Een verhoging van de microbiële activiteit in het darmkanaal zou de hydrolyse en absorptie van nutriënten verlagen. Met name de vetvertering zou nadelig worden beïnvloed doordat de microflora in staat zijn galzouten te transformeren, terwijl de eiwit- en zetmeelvertering nadelig zouden worden beïnvloed door een verhoogde competitie tussen gastheer en microflora. Tevens zou de absorptie van nutriënten kunnen worden verlaagd vanwege een verandering van de morfologie van de darmwand.

Het doel van het onderzoek beschreven in **Hoofdstuk 2** was gericht op het bestuderen van de effecten van een endo-xylanase enzym preparaat in een tarwe/rogge rantsoen op de produktieresultaten en verteerbaarheid van nutriënten in relatie tot de vetbron in het rantsoen. Als vetbronnen werden destruktievet en sojaolie in het onderzoek betrokken. Het opnemen van endo-xylanase in het rantsoen met sojaolie had slechts een gering positief effect op de produktieresultaten en de verteerbaarheid van nutriënten. Daarentegen resulteerde het toevoegen van een endo-xylanase aan het rantsoen met destruktievet in een significante verbetering van de groei, voederconversie en verteerbaarheid van nutriënten, N retentie en metaboliseerbare energie waarde van het rantsoen. De verbetering in vetvertering was daarbij het meest uitgesproken. Op basis van de resultaten van dit onderzoek werd geconcludeerd dat de effecten van een endo-xylanase enzym preparaat in een tarwe/rogge rantsoen op de produktieresultaten en de verteerbaarheid van nutriënten in belangrijke mate worden bepaald door het type vet in het rantsoen. Verder duidden de resultaten van dit onderzoek er op dat mogelijk andere factoren anders dan de ileale viscositeit verantwoordelijk zijn voor de anti-nutritionele eigenschappen van wateroplosbare NSP.

In **Hoofdstuk 3** worden de resultaten van een onderzoek beschreven dat was gericht op het vaststellen van de effecten van hoogveresterde citrus pectine (HMC), laagveresterde citrus pectine (LMC) en hoogveresterde suikerbieten pectine (HMS) op de produktieresultaten en fysiologische kenmerken bij vleeskuikens. De drie pectine produkten werden in doseringen van 15 en 30 g/kg opgenomen in een rantsoen dat was gebaseerd op maïs en soja. Het opnemen van LMC of HMS in het rantsoen had vrijwel geen invloed op de produktieresultaten van vleeskuikens. Toevoeging van HMC aan het rantsoen resulteerde daarentegen in een significante verslechtering van de groei en de voederconversie. Het effect van HMC op de groei en voederconversie was dosis afhankelijk. Wateropname en water/voer verhouding waren verhoogd na het toevoegen van één van de drie pectine produkten in het rantsoen. Daarbij was het effect van HMC op de wateropname en de water/voer verhouding het grootst. De *in vitro* viscositeit van het rantsoen was verhoogd na het opnemen van LMC of HMC in het rantsoen, terwijl HMS hierop vrijwel geen invloed had. Het waterbindend vermogen van het rantsoen en van de excreta bleek significant te worden verhoogd na het opnemen van één van de drie pectine produkten in het rantsoen. Het opnemen van HMC in het rantsoen verlaagde de concentratie aan vluchtige vetzuren (VVZ) in de blinde darmen, terwijl LMC hierop geen invloed had. Daarentegen bleek de concentratie aan VVZ in de blinde darmen van kuikens gevoerd op de HMS rantsoenen te worden verhoogd. Op basis van de resultaten van dit onderzoek werd geconcludeerd dat het effect van pectine op de produktieresultaten van vleeskuikens afhankelijk is van het percentage veresterde carboxyl groepen, de herkomst van het pectine produkt en de dosering aan pectine in het rantsoen.

In **Hoofdstuk 4** zijn de resultaten gerapporteerd van een onderzoek met vleeskuikens, waarin de effecten van HMC en LMC op de produktieresultaten, de verteerbaarheid van nutriënten, de morfologie van de dunne darmwand en de microbiële activiteit in het ileum werden geëvalueerd. Beide pectine produkten werden getest bij een dosering van 30 g/kg in een rantsoen dat was gebaseerd op maïs en soja. Het opnemen van HMC in het rantsoen verslechterde de produktieresultaten en verlaagde de verteerbaarheid van de nutriënten, met name die van vet. Toevoeging van LMC aan het rantsoen had vrijwel geen negatieve invloed op de produktieresultaten, alleen de vetvertering was verlaagd. Het opnemen van HMC of LMC in het rantsoen verhoogde de viscositeit van de dunne darminhoud. De microbiële activiteit in het ileum, met name die van de *Enterococci*, *Bacteroidaceae*, *Clostridia* en *Escherichia coli*, bleek te worden verhoogd na een toevoeging van HMC aan het rantsoen. Daarnaast onderging de morfologie van de darmwand een sterke verandering bij het opnemen van HMC in het rantsoen. Verder bleek bij deze dieren het aantal slijmbekercellen per 100 villi cellen en de sucrase isomaltase activiteit te worden verhoogd. Het opnemen van LMC in het rantsoen had vrijwel geen invloed op de microbiële activiteit in het ileum, enkel het aantal *Clostridia* was verhoogd. Ook de morfologie van de darmwand bleek nauwelijks te worden beïnvloed door LMC, terwijl het aantal slijmbekercellen per 100 villi cellen wel significant was verhoogd. Op basis van de resultaten van dit onderzoek werd geconcludeerd dat de anti-nutritionele effecten van wateroplosbare pectinen geassocieerd lijken te zijn met veranderingen in het ecosysteem in het ileum en met veranderingen in de morfologie en histologie van de darmwand in het ileum.

Hoofdstuk 5 beschrijft een onderzoek waarin de effecten van HMC, LMC, tarwe en rogge op de activiteit en samenstelling van de microflora en de mate van galzout deconjugatie in de dunne darm in relatie tot de vetbron in het rantsoen bij vleeskuikens werden onderzocht. Sojaolie en destruktievet waren de vetbronnen die in het onderzoek werden gebruikt. Het opnemen van HMC, LMC of rogge/tarwe in het rantsoen resulteerde in een significante verslechtering van de produktieresultaten van vleeskuikens. Dit negatieve effect van HMC, LMC en rogge/tarwe op de produktieresultaten was groter in het rantsoen met destruktievet dan in het rantsoen met sojaolie. De toevoeging van HMC, LMC of rogge/tarwe aan het rantsoen verlaagde de pH van de darminhoud in het ileum, terwijl de concentratie aan VVZ in de darminhoud van het ileum was verhoogd bij de dieren gevoerd op het rogge/tarwe rantsoen. Het opnemen van HMC in het rantsoen verhoogde het totaal aantal aërobe en anaërobe bacteriën alsmede het aantal *Bifidobacteriën*, *Enterococcen* en *E. coli*. Bij kuikens gevoerd op het rogge/tarwe rantsoen werd alleen een significante verhoging van het aantal *E. coli* bacteriën vastgesteld. Het opnemen van LMC in het rantsoen had vrijwel geen invloed op de microbiële activiteit en samenstelling. De concentraties aan geconjugeerde galzouten werden significant verlaagd bij het opnemen van HMC, LMC of rogge/tarwe in het rantsoen, terwijl de corresponderende concentraties aan ongeconjugeerde galzouten werden verhoogd. Uit de resultaten van dit onderzoek werd geconcludeerd dat, naast een verhoging van de viscositeit van de dunne darminhoud, een toename in de microbiële activiteit in de dunne darm ten minste ten dele kan worden geassocieerd met de anti-nutritionele eigenschappen van wateroplosbare NSP.

Het doel van het onderzoek beschreven in **Hoofdstuk 6** was er op gericht meer inzicht te verkrijgen over de invloed van de microflora in het darmkanaal op de anti-nutritionele eigenschappen van wateroplosbare NSP. Hierbij werd gebruik gemaakt van conventionele en kiemvrije vleeskuikens. De wateroplosbare NSP bevattende produkten betrokken in het onderzoek waren HMC en rogge. Het verstrekken van het HMC of het rogge rantsoen aan conventionele kuikens resulteerde in een significant slechtere groei en voederconversie. Daarentegen werd bij het verstrekken van deze rantsoenen aan kiemvrije kuikens alleen de groei op het HMC-rantsoen significant verlaagd. Het verstrekken van het HMC-rantsoen aan conventionele kuikens verlaagde de energie- en zetmeel verteerbaarheid aan het eind van het ileum. De faecale verteerbaarheid van nutriënten, de N retentie en de metaboliseerbare energiewaarde van het rantsoen werden significant verlaagd bij het verstrekken van het HMC-rantsoen aan conventionele kuikens. Het verstrekken van het HMC-rantsoen aan kiemvrije kuikens had vrijwel geen invloed op de faecale vertering van de nutriënten en de N retentie, terwijl de metaboliseerbare energiewaarde van het rantsoen was verhoogd. Het verstrekken van het HMC- of het rogge rantsoen aan conventionele of kiemvrije kuikens verhoogde de viscositeit van de dunne darminhoud. Deze verhoging in viscositeit van de dunne darminhoud was aanzienlijk groter in conventionele kuikens dan in kiemvrije kuikens. Het verstrekken van het HMC- of het rogge rantsoen aan conventionele kuikens resulteerde in een sterke verandering van de morfologie van de darmwand van het ileum, terwijl bij kiemvrije kuikens de morfologie van de dunne darmwand nauwelijks was beïnvloed. Op basis van de resultaten van dit onderzoek werd geconcludeerd dat de microflora in het maagdarmkanaal de mate van de anti-nutritionele effecten van wateroplosbare NSP in vleeskuikens medieert middels een beïnvloeding van de viscositeit en de fermentatie van de wateroplosbare NSP in het maagdarmkanaal.

In de **Algemene discussie** werden de uitkomsten van de studies tezamen met de literatuur nader geëvalueerd en werden enkele praktische implicaties bediscussieerd. De belangrijkste conclusies van de studies beschreven in dit proefschrift kunnen als volgt worden samengevat:

- Het opnemen van wateroplosbare NSP in vleeskuikenvoeders verhoogt de microbiële activiteit en verandert de microbiële samenstelling in het ileum. Deze verhoging in microbiële activiteit en verandering in microbiële samenstelling medieert de mate van de anti-nutritionele effecten van de wateroplosbare NSP fracties in vleeskuikens middels een beïnvloeding van de viscositeit en de fermentatie van wateroplosbare NSP in het maagdarmkanaal. .
- De verhoging in microbiële activiteit bij het opnemen van wateroplosbare NSP in het rantsoen stimuleert deconjugatie van galzouten in het ileum van vleeskuikens. Deze verhoging in galzout deconjugatie zou voor een deel de reductie in vetvertering kunnen verklaren bij aanwezigheid van wateroplosbare NSP in het rantsoen van vleeskuikens.
- De verhoging in microbiële activiteit en de verandering in microbiële samenstelling in het ileum van vleeskuikens bij het verstrekken van rantsoenen met wateroplosbare NSP resulteert in een verandering van de morfologie van de darmwand. Deze verandering van de darmwand kan de absorptie van nutriënten beïnvloeden.
- De verhoging in de viscositeit van de dunne darminhoud is niet de enige verklarende factor voor de anti-nutritionele effecten van wateroplosbare NSP bij vleeskuikens.

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Het proefschrift dat voor u ligt is het resultaat van een onderzoek dat grotendeels is uitgevoerd op de afdeling Diervoeding en Fysiologie (ILOB) van TNO-Voeding. Bij het onderzoek waren verschillende disciplines betrokken. Deze multidisciplinaire aanpak had tot gevolg dat ik bij de voorbereiding en de uitvoering van het onderzoek vaak een beroep heb moeten doen op de medewerking van vele mensen. Het is dan ook zeer plezierig dat ik overal welwillende mensen ben tegengekomen die me uitstekend hebben geholpen. Gaarne maak ik van de gelegenheid gebruik om iedereen van harte te bedanken voor de bereidwillige medewerking. Een aantal mensen wil ik daarbij in het bijzonder noemen.

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Pim

Curriculum vitae

Dirk Jacob Langhout (Pim) werd op 29 oktober 1965 geboren in Nieuw Vennep, gemeente Haarlemmermeer. In 1984 behaalde hij het VWO diploma aan het Corderius College te Amersfoort. In hetzelfde jaar begon hij aan zijn studie Zoötechniek aan de Landbouwuniversiteit te Wageningen. In juni 1990 studeerde hij af met Gezondheid en ziekteleer en Veevoeding als afstudeervakken. Van oktober 1990 tot juli 1994 is hij werkzaam geweest als sektorspecialist varkens- en pluimveevoeding bij de Aan- en verkoop Coöperatie Meppel b.a. (ACM), te Meppel. Van juli 1994 tot augustus 1998 is hij werkzaam geweest als nutritionist bij de afdeling Diervoeding en Fysiologie (ILOB) te Wageningen van TNO-Voeding, waar het in dit proefschrift beschreven werk werd verricht. Vanaf 1 augustus 1998 is hij werkzaam als produkt manager pluimvee bij Provimi b.v. te Rotterdam.