Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens

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Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens

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Proefschrift

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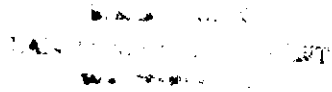

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The aim of the thesis was to identify the physicochemical properties of non-starch polysaccharides (NSPs) that are most relevant to the nutrition of the broiler chicken. More specifically, the mechanisms by which fibre viscosity can affect lipid digestibility in broiler chickens have been investigated in a series of experiments. The effect of fibre viscosity *per se* was investigated by using carboxymethylcelluloses (CMC) with varying viscosity. The CMC types were non-fermentable *in vitro*. Fibre viscosity *per se* depressed the digestibility of lipids, protein and starch in broiler chickens fed diets containing CMC with varying viscosity. The effect of fibre viscosity on lipid digestibility was dependent on the type of fat. The digestibility of animal fat that was predominantly composed of saturated long chain fatty acids was depressed, whereas there was no effect on the digestibility of soyabean oil or coconut oil. The reduction in lipid digestibility coincided with a reduced bile salt concentration and raised microbial numbers in the small intestine. CMC did not affect the condition of the small intestinal mucosa. The morphological parameters indicated that the condition of the mucosa was even improved in CMC fed birds. A study with germfree rats and rats with a specific pathogen free intestinal flora revealed that the effect of CMC on lipid digestibility is mediated, at least partially, by the intestinal flora. It was concluded that the small intestinal microflora can mediate the antinutritive effect of fibre viscosity on lipid digestibility in broiler chickens. Moreover, it was proposed that a reduction in bile salt concentration and bacterial transformation of bile salts reduces their efficacy to solubilise lipids. The results show that fibre viscosity is an important antinutritive property which should be taken into account in diet formulation for broiler chickens.

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STELLINGEN

1. De fysische eigenschappen van voedingsvezels zijn uit voedingsfysiologisch oogpunt belangrijker dan de chemische samenstelling. *Dit proefschrift.*

2. De viscositeit *per se* is een anti-nutritionele eigenschap van niet-zetmeel polysacchariden in de voeding van kuikens. *Dit proefschrift.*

3. De microflora in de dunne darm medieert het anti-nutritionele effect van visceuze voedingsvezels op de vetvertering bij kuikens. *Dit proefschrift.*

4. De bevinding dat antibiotica-toediening geassocieerd is met een hogere vetabsorptie bij zuigelingen (Verkade *et al.*, 1989) ondersteunt de hypothese dat de microflora in de dunne darm de vetabsorptie kan verlagen.
Verkade et al., 1989, Eur. J. Pediatr., 149: 126-129.

5. Het slijm in het maagdarmkanaal is van levensbelang. Visceuze voedingsvezels kunnen daaraan een positieve bijdrage leveren.

6. De microflora in de dunne darm is met betrekking tot de nutriënten-voorziening van de gastheer meer een parasiet dan een symbiont.

7. Vet- en cholesterolarme zuivelprodukten met oplosbare, visceuze cellulose-ethers dragen bij tot een verantwoord bloedcholesterolgehalte.

8. Voor het toepasbaar maken van resultaten uit wetenschappelijk onderzoek is de benadering 'Keep It Simple and Stupid' zeer zinvol.

9. Landbouwers verdienen een positief imago, zij zijn immers de hoveniers van het Nederlandse landschap.

10. De uitspraak 'wat ben je toch een varken' is gezien de fysiologische overeenkomsten tussen mens en varken voor meer dan 95% niet te verwerpen en dus significant.

C.H.M. Smits
Stellingen behorende bij het proefschrift
'Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens'
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Voorwoord

Het proefschrift dat voor u ligt is grotendeels het resultaat van ruim vijf jaar onderzoek op het Instituut voor de Veevoeding 'De Schothorst'. Het doel was na te gaan welke fysisch-chemische eigenschappen van niet-zetmeel koolhydraten van belang zijn voor de vertering en voor de gezondheid. Daarbij werd het kuiken al vrij snel als modeldier gekozen. Het proefschrift werd vervolgens het resultaat van een multidisciplinaire aanpak, zoals dat met een mooi woord heet, en dat betekent dat vele mensen een bijdrage hebben geleverd aan het onderzoek.

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microbiologische bepalingen en hebben de proef met kiemvrije ratten uitgevoerd. Hen allen wil ik hiervoor bedanken.

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Dr. Harry Vahl (ACM), dr. Ben Schutte (ILOB-TNO) en Henk Sloetjes (ID-DLO) ben ik erkentelijk voor hun bijdrage aan de oriënterende studie met kiemvrije kuikens.

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Contents

Chapter 1

Scope, aim and outline of thesis

Scope, aim and outline of thesis

In farm animal nutrition, diets are formulated on the basis of the nutritive value of the ingredients and their costs. The diets are defined according to nutritional specifications that meet the requirement of the target species. However, there are constraints in relation to undesirable effects such as intake and digestion problems. The constraints may limit the use or content of certain feedstuffs. Feedstuffs may contain one or more undesirable factors and within feedstuffs the level of that factor may vary. Examples are anti-nutritional factors like trypsin inhibitors, lectins, tannins, alkaloids and glucosinolates (Huisman & Tolman, 1992). These critical factors are well defined, can be determined in the laboratory and the nutritionist can take them into account in evaluating the quality of the ingredients and in diet formulation.

The role of dietary fibre is complex. Dietary fibre has been defined as the fraction of plant material that is resistant to hydrolysis by endogenous enzymes of the digestive tract (Hipsley, 1953). Although there is still debate about this definition, it is clear that dietary fibres represent a wide variety of chemical substances with a tremendous range of physicochemical properties (Roberfroid, 1993). They vary in chemical structure, physical structure, solubility, water holding capacity, viscosity, cation exchange capacity, adsorptive properties etc.. It is well established in human nutrition that certain physicochemical properties of dietary fibres may be beneficial for human health. Low intakes of fibre are linked to chronic disorders such as constipation, diverticulitis and cancer of the large bowel as well as risk of obesity, cardiovascular disease and diabetes (Burkitt & Trowell, 1975).

In the nutrition of monogastric farm animals, however, little attention has been paid to physicochemical properties of fibres in relation to the process of digestion and absorption of nutrients, the intestinal microflora and health. The nutritional evaluation of dietary fibre generally focuses on the nutritive value of the fermentable fibre fraction, the other aspects being neglected and the undigested portion is considered as inert non-nutritive material. Recently, the physicochemical properties of fibre have received more attention in animal nutrition, particularly in poultry nutrition. However, in contrast to human nutrition, the physicochemical properties of fibres had been associated with detrimental rather than with beneficial effects. The ingestion of diets rich in gelling non-starch polysaccharides (NSPs) from wheat, barley and rye, reduced

the digestibility of protein, starch and lipids in broiler chickens (Antoniou *et al.*, 1981; Choct and Annison, 1990; Fengler and Marquardt, 1988; White *et al.*, 1981). This 'antinutritive' effect was associated with an increase in digesta viscosity. In practice, the problem of gelling NSPs can be solved, at least partially, by the addition of NSP degrading enzymes to broiler chicken diets (Bedford *et al.*, 1991; Petterson & Aman, 1988; Veldman & Vahl, 1994). It has been shown that NSP-ases reduce digesta viscosity and elevate nutrient digestibility. However, the mechanism by which gelling NSPs affect nutrient digestibility in broiler chickens has not been elucidated and thus the most critical properties of gelling NSPs are unknown. Ideally, these critical properties should be taken into account in diet formulation.

This thesis attempts to identify the physicochemical properties of NSPs that are most relevant for poultry nutrition. More specifically, experiments were carried out to unravel the mechanism by which the model high viscosity compound, carboxymethyl cellulose (CMC), reduces lipid digestibility.

A literature review was conducted to describe the NSPs present in plant ingredients and to identify the most critical physicochemical properties and their effects on nutrient digestibility (Chapter 2). It was concluded that the viscosity of NSPs is the most relevant property. The process of lipid digestibility was then selected to describe the mechanisms by which gelling NSPs may depress feed utilization. It was noted that the microflora may play a key role in the anti-nutritive effect of gelling NSPs. The effect of fibre viscosity *per se* on macronutrient digestion was examined in broiler chickens by using CMC of varying viscosity (Chapter 3). Subsequently, the inhibitory effect of CMC on lipid digestibility was further studied by using different fat types (Chapter 4). To obtain more insight into the small intestinal modifications induced by fibre viscosity, the condition of the mucosa (Chapter 5) and the bile salt concentration and microflora (Chapter 6) were examined in the small intestine of broiler chickens. The role of the microflora in the inhibitory effect of fibre viscosity on lipid digestibility was investigated in germfree rats and rats with a normal flora (Chapter 7). Finally, the outline of a system is proposed for the dietary evaluation of carbohydrates, in which the critical anti-nutritive properties of fibres are taken into account (Chapter 8).

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Chapter 2

Non-starch plant polysaccharides in broiler nutrition - Towards a physiologically valid approach to their determination

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Non-starch plant polysaccharides in broiler nutrition - Towards a physiologically valid approach to their determination

Coen H.M. Smits* and Geoffrey Annison

Abstract

The physicochemical properties of non-starch polysaccharides (NSPs) are responsible for their antinutritive activities in the broiler chicken. In particular soluble viscous NSPs depress the digestibilities of protein, starch and fat. On the other hand, insoluble and non-viscous NSPs may have a beneficial effect. Fat digestion is used here to illustrate how the physicochemical properties of NSPs may interfere with digestion and absorption. It is suggested that the gut microflora can mediate the anti-nutritive effects of soluble and viscous NSPs. It is concluded that the determinations of crude fibre and/or acid detergent fibre and neutral detergent fibre in feedstuffs, are not appropriate for predicting and understanding the physiological action of NSPs in broilers. The determination of the *in vitro* solubility of NSPs and the viscosity of the feed ingredient could become of major importance for 'anti-nutritional' evaluation. More research needs to be conducted to study the interactions of NSPs with the microbial activity in the intestinal tract of the broiler chicken.

* On leave from De Schothorst at the Monogastric Research Centre, Department of Animal Science, Massey University, Palmerston North, New Zealand.

Introduction

The physicochemical properties of the fibre polysaccharides in dietary plant ingredients have been recognised as being responsible for their physiological action, particularly in human nutrition. Thus, dietary fibre has been studied in relation to health problems such as hypercholesterolaemia, constipation, diverticulitis, cancer of the large bowel, obesity and diabetes. However, less attention has been paid to the consequences of the physicochemical properties of dietary fibre in farm animal nutrition. This topic has recently received more

attention in poultry nutrition as a result of the recognition of the 'antinutritive' properties of some non-starch polysaccharides (NSPs) present in rye (Antoniou et *al.*, 1981; Campbell *et al.*, 1983b; Fengler and Marquardt, 1988; Bedford *et al.*, 1991), wheat (Choct and Annison, 1990) and barley (White *et al.*, 1981; Campbell *et al.*, 1989; Wang *et al.*, 1992). Annison (1993) has described a possible mechanism whereby cereal NSPs can influence the digestive processes in chicks, and has emphasised the importance of the solubility and the viscosity of the NSP fraction. One of his conclusions was that ascribing the antinutritive effects solely to the increased viscosity of the digesta may be too simplistic. Other physicochemical characteristics of NSPs and dietary ingredients may play a role in the overall effect. The aim of this paper is to identify the physicochemical properties of NSPs most relevant to the nutrition of the chicken and to propose a mechanism whereby these properties may influence the digestibility of fat in broilers. Fat digestion is used as an example to illustrate the complexity of the interaction between NSPs and dietary nutrients. Finally, the physicochemical evaluation of dietary NSPs are discussed. By way of introduction, the chemical structures of NSPs and how polysaccharides interact to form the plant cell wall, are briefly reviewed.

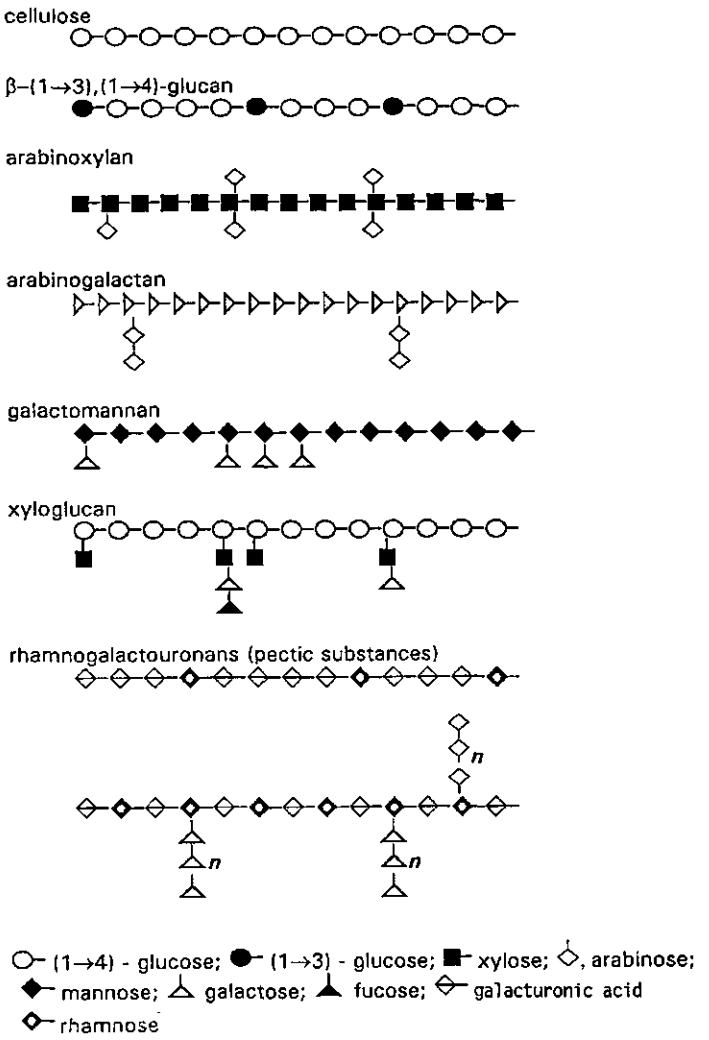
Physicochemistry of non-starch plant polysaccharides

Chemical structures

Polysaccharides consist of macromolecular polymers of simple sugars or monosaccharides. The sugars are joined by a specific type of linkage called a glycosidic bond which is formed between the hemi-acetal (or hemiketal group) of one sugar and the hydroxyl group of another. The number of possible glycosidic bonds between monosaccharides is five for a hexose and four for a pentose. They are identified by referring to the carbon atoms of each sugar which are involved in the bond and by the orientation of the hemiacetal oxygen atom (α- or β-). In starch the glucose molecules are joined mainly by α-(1→4) bonds with a small number of α-(1→6) bonds. These bonds and the α-(1→2) link between glucose and fructose in sucrose, the β-(1→4) link between glucose and galactose in lactose and the α-(1→1) link between the glucose units of trehalose are cleaved by endogenous avian or mammalian enzymes. All other glycosidic bonds are resistant to digestive enzymes but they may be cleaved by microbially derived enzymes. NSPs contain sugars other than glucose and have glycosidic

bonds other than the α-(1→4), α-(1→6) bonds present in starch. The importance of the nature of the bonds in determining their susceptibility to cleavage by avian digestive enzymes is illustrated by the resistance of cellulose (a β-(1→4) glucan) to starch-degrading enzymes.

Figure 1 Polysaccharide structures commonly found in feed ingredients of plant orgin.



The majority of NSPs in poultry diets is of plant origin and in modern formulations the variation in both the amount and structure may be large. This reflects the diversity of the ingredients which are currently available to the compound animal feed trade. Many different types of monosaccharides may be present **(Figure 1)** and these may form many different structures. The polysaccharides may be relatively simple, such as the cereal β-D-glucans which are linear polymers of glucose with β-(1→3),(1→4) glycosidic links **(Figure 2)**, (Fincher and Stone, 1986). The other major cereal cell wall polysaccharides, the arabinoxylans, are more complex **(Figure 3)** being composed of two sugars, arabinose and xylose in a branched structure (Hoffmann, 1991, Annison *et al.*, 1992). Even more complex polysaccharides may be present in legumes. The main NSP of lupins is a polymer with a highly complex branched structure containing long β-(1→4)-D-galactose side chains attached to a pectin-like main chain consisting of rhamnose and galacturonic acid linked by β-(1→4) and α-(1→2) bonds respectively. There are also side chains of α-(1→5)-L-arabinose, (Cheung, 1991).

Figure 2 Major soluble non-starch polysaccharide of barley: ß-(1→3),(1→4)-D-glucan.

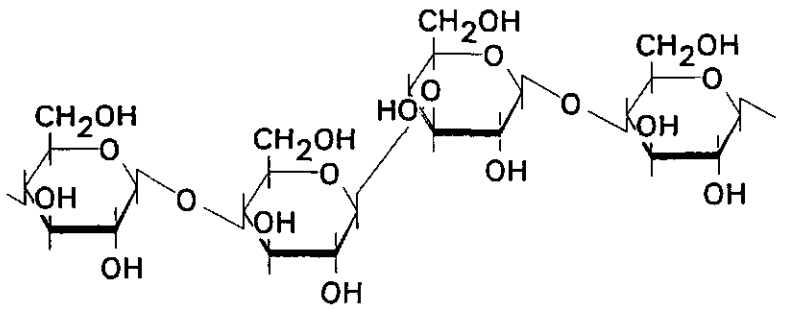
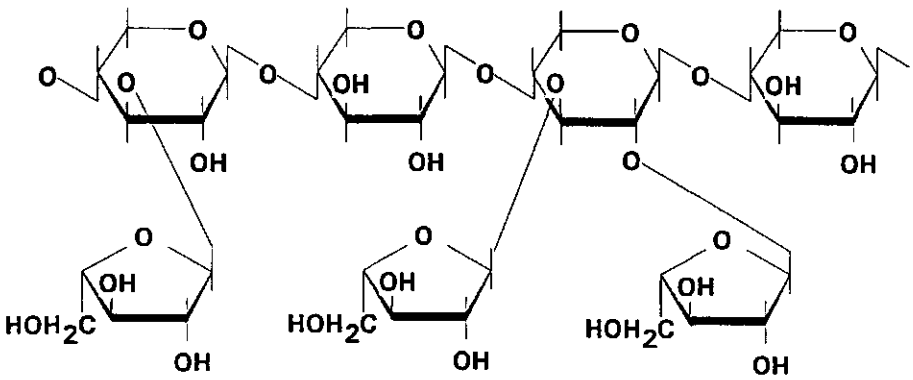


Figure 3 Major soluble non-starch polysaccharide of rye and wheat: arabinoxylan



Plant cell wall structure

The great range in the chemical structures of NSPs means that they have different physical properties. These will be discussed later. It is important to emphasize, however, that NSPs do not generally exist as completely separate components in feedstuffs. Most NSPs are part of the cell wall and are closely associated with other polysaccharides or non-carbohydrate material such as protein and lignin (Fincher and Stone 1986; Selvendran *et al.*, 1987). These associations must be considered because it is likely that they will influence the manner in which the NSPs behave when ingested. Solubility is an important property which determines the antinutritive activity of NSPs in broiler diets (Annison, 1993). The solubilities of NSPs are determined not only by their primary structure, but also by how they are bound to other cell wall components.

Plant cell walls are highly ordered and consist of different polysaccharides, polyphenols, glycoproteins and glycolipids. The components are arranged in three main patterns to give fibrillar polysaccharides (mainly cellulose), matrix polysaccharides (mainly hemicelluloses and pectin) and encrusting substances (mainly lignin). The concentrations of these components vary between different plants and between different plant parts and are also influenced by the degree of

plant maturity. The structures of plant cell walls has been reviewed in detail by Selvendran *et al.*, (1987). The structural arrangements within the cell wall will also affect the physical and chemical properties of the polysaccharides and these may in turn affect their physiological action.

A second level of association is the bonding between molecules of the cell wall components. There may be weak hydrogen bonding, as well as stronger ionic cross-linking through cations or covalent bonds. The arabinoxylans of wheat, for example, are thought to be linked to lignin through a phenolic acid ester bond. The arabinoxylans are also closely associated with wheat proteins and there is strong evidence that they are linked covalently. Fincher *et al.*, (1974) isolated a covalently linked arabinogalactan peptide from wheat endosperm. The binding of the polysaccharides to other food components is of importance because of its influence on the solubility of the polysaccharide in aqueous media.

Viscosity and water holding capacity

Many polysaccharides when dissolved in water give viscous solutions. Viscosity is dependent on several factors including the size of the molecule, whether it is branched or linear, the presence of charged groups, the surrounding structures and, of course, the concentration. Polysaccharides increase viscosity at low concentrations by directly interacting with the water molecules. As the concentration increases the molecules of the polysaccharide themselves interact and become entangled in a network (Morris and Ross-Murphy, 1981). This process can cause great increases in the viscosity and is dependent on the formation of junction zones between the polysaccharide molecules. Gel formation can occur when the interactions of the polysaccharide molecules become great. Because of the formation of networks with water the viscosities and water holding capacities of soluble NSPs are relatively high compared with those of insoluble NSPs. Insoluble polysaccharides such as cellulose and xylans can hold water as they behave like sponges but their viscosities are relatively low.

Binding of small ions and molecules and surface activity

Some NSPs, such as the pectins may have a high charge density at given pH values because of the presence of acidic groups. Apart from the association of

cations with the negatively charged groups, in some NSPs the three-dimensional structure of the molecule allows a chelation of ions to occur. Indeed, cations can form ionic bridges between NSP molecules and profoundly influence their viscosities and gel forming properties. Although it has not yet been investigated, the possibility of cations acting as ion bridges between polysaccharides and small charged molecules should not be discounted. Small molecules may also be bound weakly to polysaccharides through both hydrophobic and hydrophillic bond interactions.

NSP may also have a surface activity. Polysaccharides can present charged (negative and, less commonly, positive) as well as weakly hydrophobic and weakly hydrophillic surfaces. When in solution they tend therefore to associate with surfaces. For example, after ingestion these may be the surfaces of food particles, the surface of lipid micelles or the glycocalyx surface of the gut.

Physiological effects of NSPs

Because of the importance of the complex interactions between the different chemical components present in plant tissues, it makes little sense, nutritionally, to describe dietary fibre solely in chemical terms. Rather, it may be better to describe the cell wall polysaccharide components of feedstuffs in terms of their physicochemical properties, which are likely to be related to their physiological effects.

It has been demonstrated in several laboratories that the addition of certain NSPs to broiler diets affect the birds ability to digest starch, protein and lipids **(Table 1)**. It can be seen that lipid digestibility is particularly depressed by NSPs. Therefore, the process of fat digestion may be an appropriate mode to highlight how the physicochemical properties of NSPs affect the digestive processes.

The process of fat digestion in monogastric animals has recently been reviewed by several authors (Freeman, 1976; Davidson and Glickman, 1983; Freeman, 1984; Bezard and Buguat, 1986; Stremmel, 1987). Limited information, however, is given as to how the physicochemical properties of NSPs might affect fat digestion in poultry. In order to put forward a mechanism by which NSPs may affect fat digestion in broiler chickens, it is therefore necessary to use

Table 1 Effect of isolated soluble plant non-starch polysaccharides (NSPs) on the digestibility of starch, protein and lipids in broiler chickens.

Reference	Dietary treatment	total pentosan g/kg DM	Digestibility Starch	Protein	Lipid	
Choct and Annison (1992a)	Control diet	25.9	0.96	0.75	0.93	
	+ 20 g/kg DM water-extracted pentosan	43.8	0.91	0.70	0.87	
	+ 5 g/kg DM alkali-extracted pentosan	30.8	0.96	0.75	0.93	
	+ 10 g/kg DM alkali-extracted pentosan	34.9	0.95	0.73	0.92	
	+ 25 g/kg DM alkali-extracted pentosan	48.0	0.92	0.69	0.76	
	+ 40 g/kg DM alkali-extracted pentosan	65.7	0.82	0.61	0.69	
					C18:0	C18:2
Choct and Annison (1992b)	Control diet		0.98	0.69	0.71	0.96
	+30 g/kg pentosan		0.92	0.63	0.41	0.82
	+30 g/kg pentoses (15 g/kg arabinose + 15 g/kg xylose)		0.98	0.72	0.66	0.95
	+ 30 g/kg depolymerized pentosan		0.94	0.69	0.70	0.96
Fengler and Marquardt (1988)	Experiment I					
	wheat based diet (52 %)				0.76	
	wheat based diet + 15 g/kg crude pentosan[1]				0.74	
	wheat based diet + 30 g/kg crude pentosan				0.60	
	wheat based diet + 60 g/kg crude pentosan				0.51	
	Experiment II					
	wheat based diet (57.5 %)				0.75	
	wheat based + 19 g/kg crude pentosan				0.65	

[1] Crude pentosan: arabinose + xylose = 42 % DM

information obtained from *in vitro* studies and *in vivo* studies with pigs, rats and humans.

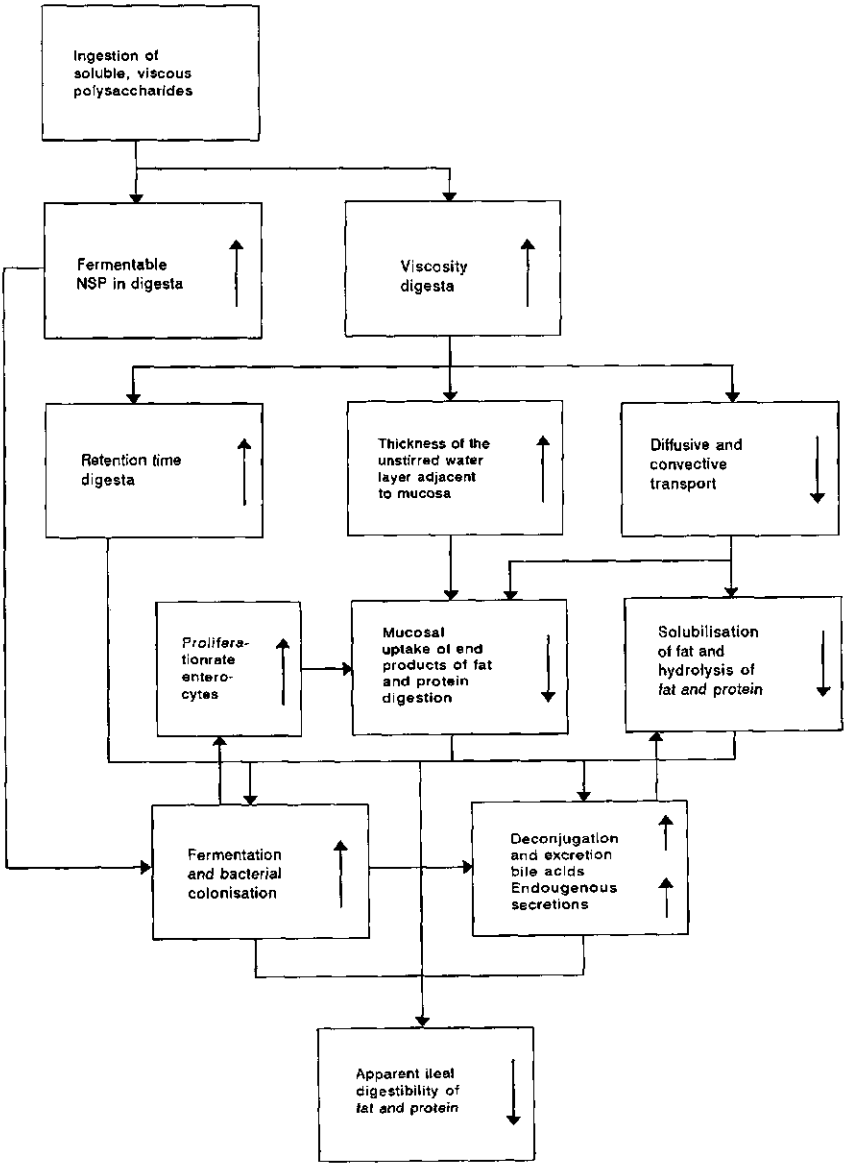
Physiological effects of viscosity and water holding capacity of soluble NSPs

It has been noted that the ingestion of soluble, viscous NSP is associated with an increase in digesta viscosity in broiler chickens (White *et al.*, 1983; Rotter *et al.*, 1989; Bedford *et al.*, 1991; Choct and Annison, 1992b). However, the mechanism by which viscosity affects the digestion of lipids is presently not known. Nevertheless, there is sufficient published information available to describe important antinutritive physiological effects that may be a consequence of viscosity. These effects are presented in **Figure 4.**

The viscous properties can impair the diffusion and convective transport of

lipase, oils and bile salt micelles within the gastrointestinal contents. Edwards *et al.* (1988) demonstrated *in vitro* that the convective transport of glucose and sodium was impaired in a viscous environment. Moreover, viscosity may reduce the contact intensity between potential nutrients (e.g. fats) and the digestive secretions (e.g. lipases, bile salts), and impair transport to the epithelial surface. Isaksson *et al.* (1982) have shown *in vitro* that, in a viscous environment, the activities of lipase and other enzymes are reduced. However, in contrast to these findings, *in vivo* studies with rats fed on viscous polysaccharides have shown that the activity of enzymes in the gastrointestinal tract is increased rather than reduced after the ingestion of gelling agents (Ikegami *et al.*, 1990; Poksay and Schneeman, 1983). This may have been caused by increases in pancreatic-biliary secretions. Moreover, the recent work of Larsen *et al.* (1994), who examined the effects of fibre viscosity *per se* on the proteolytic enzyme activities in the chyme of the rat using low fermentable carboxymethylcellulose with different viscosities, has shown that there was no significant effect of viscosity on enzyme activity. It therefore seems unlikely that a reduction in lipase activity limits fat digestion in viscous digesta. On the other hand, there is evidence that the low bile acid concentration in the intestinal tracts of young broilers limits fat digestion. Bile acids, which are necessary to emulsify the water insoluble fat components, promote lipase activity by increasing the active surface and subsequently enhance mixed micelle formation of monoacylglycerides, free fatty acids, cholesterol and fat-soluble vitamins. Although the broiler chick has a reserve of bile acids in the yolk sac after hatching, the concentration of bile acids in the gastrointestinal tract is relatively low in the first weeks of life (Green and Kellog, 1987; Iñarrea *et al.*, 1989). Supplementation of broiler diets with bile acids significantly improved fat digestion (Edwards, 1962; Gomez and Polin, 1974, 1976; Polin *et al.* 1980; Polin and Hussein, 1982; Kussaibati *et al.*, 1982a). Kussaibati *et al.* (1982b) noted an improvement in fat digestibility in germfree and conventional broiler chicks fed on diets supplemented with bile acids and concluded that the response to bile acids is not entirely associated with an active microflora. According to Ebihara and Schneeman (1983), viscous NSPs may be able to entrap bile salts and thereby reduce their effectiveness in solubilising the fat components and subsequently lipid absorption. Campbell *et al.* (1983a) demonstrated that the addition of sodium taurocholate to a rye based diet substantially improved the digestibility of lipids by broiler chickens. Likewise, the lipid digestibility from a wheat-based diet was also improved by bile salt addition, but the magnitude of the improvement was less pronounced.

Figure 4 Effects of soluble, viscous non-starch polysaccharides (NSPs) that may be associated with the detrimental effect on the digestion of fat and protein in the small intestine of broiler chickens.



For absorption to take place, the fatty acids and the monoacylglycerols must cross an aqueous barrier, the unstirred water layer (UWL) which is adjacent to the intestinal mucosa (Wilson *et al.*, 1971; Wilson and Dietschy, 1974). It has been shown that gel-forming gums and pectin give rise to an increase in the thickness of the UWL (Johnson and Gee, 1981, Flourie *et al.*, 1984). According to Borgström *et al.*, (1957) and Smithson *et al.* (1981) the mucus produced by goblet cells participates in the formation of the UWL by increasing the volume of the adherent mucosal fluid and its viscosity. According to Satchithanandam *et al.* (1990) dietary fibre may increase the secretion of mucus. Larsen *et al.* (1993) reported that rats fed on a diet containing highly viscous carboxymethylcellulose had a higher concentration of sialic acids relative to chromic oxide in the small intestinal contents than rats fed on a diet containing an equal amount of a low viscosity carboxymethylcellulose. This indicates that NSP viscosity induces a secretory response of mucus. It may increase the resistance for transport of nutrients through the UWL adjacent to the epithelial surface by increasing the thickness of the mucus layer and/or by changing the physicochemical properties of the mucus.

Additionally, absorption may be affected by an increase in proliferation rate of the enterocytes and a change in the morphology of the villi and microvilli. Feeding rats various gelling agents increased the proliferation rate of the enterocytes of the jejunum and distal ileum and decreased the activity of specific epithelial surface enzymes (Johnson *et al.*, 1984, Johnson and Gee, 1986). It has been demonstrated by Stremmel *et al.* (1985) in rats that the microvilli membranes contain a fatty acid binding protein (FABP) which is involved in the absorption of fatty acids (Potter *et al.*, 1989). The absorption of fatty acids may be impaired by gelling agents if the effective surface of the microvilli and/or the functions of FABP in the mucosal membrane are diminished.

Finally, increased endogenous losses caused by viscous NSP may also result in a decrease in the apparent digestibility of fat and nitrogen at the end of the ileum. Larsen *et al.* (1993) noted that the true digestibility of protein at the terminal ileum of rats was not affected by fibre viscosity. However, the endogenous nitrogen-loss was significantly increased with increasing fibre viscosity. It is also possible that endogenous lipid losses result in impaired apparent digestibility of fat in broilers. Contrary to that of mammals, the bile of the broiler chicken contains a large amount of triacylglycerols and cholesterol esters (Noble and Conner, 1984; Noble *et al.*, 1988). Ligation of the bile duct of chickens resulted in a 2-3 fold increase in the plasma lipid concentration (Cherry, 1972 (cited by

Cherry and Jones, 1982)) which suggests that appreciable amounts of lipids are carried in the enterohepatic cycle. Larsen *et al.* (1993) showed that the extra endogenous nitrogen secretion found in rats was derived from mucus. An increased pancreatic-biliary secretion by gelling NSP may therefore only partially contribute to increased losses of endogenous lipid and nitrogen.

In summary, the decrease in apparent fat digestibility induced by NSPs with viscous properties may be caused by a reduced fat emulsification or micelle formation, by a reduced mucosal uptake of lipids and by an increased amount of unabsorbed endogenous fat in the chyme.

Interaction between viscosity and microflora

The primary cause of the decrease in fat digestibility when NSPs are fed seems to be related to their viscosity. However, it is important to emphasize that the microflora may, at least partially, be indirectly responsible for the detrimental effects. Choct *et al.* (1992) reported that the deleterious effect of wheat pentosans on the digestibility of long chain fatty acids was less pronounced in caecectomised chickens than in intact chickens. Other workers have reported pronounced improvements in the performance of broiler chickens after supplementation of rye-based diets with antibiotics, whereas the magnitude of the improvement by antibiotic supplementation was less with the control diet (Misir and Marquardt, 1978). Elwinger and Teglöf (1991) demonstrated a significant interaction between an enzyme preparation and antibiotic supplementation in a barley-based diet fed to broiler chickens. In diets without antibiotic the enzyme preparation significantly improved growth rate and feed conversion ratio, whereas in diets supplemented with antibiotic the enzyme preparation had no significant effect on the performance of the birds. The effect of fibre viscosity *per se* (carboxymethylcellulose) on fat digestion in germfree broilers has recently been investigated directly (C.H.M. Smits and H.A. Vahl, unpublished data). The broiler chickens were given either a semi-synthetic control diet or this diet to which 0.5 or 1.0% carboxymethylcellulose had been added. The results of this study are presented in **Table 2**. Although the study did not involve conventional broiler chickens as controls, the results indicate that, in germfree chicks, fibre viscosity had negligible effects on fat digestibility. It can therefore be assumed that viscous NSPs must modify bacterial activity in order to lower fat digestibility.

Table 2 The effect of the concentration of carboxymethylcellulose (CMC) on digesta viscosity and the apparent digestibility of crude fat in germfree broiler chickens[1] (C.H.M. Smits and H.A. Vahl, unpublished data: see appendix for experimental details).

	Diet		
	0% CMC	0.5% CMC	1% CMC
Digesta viscosity (mPa.s) [2]:			
Upper small intestine	4	9	18
Lower small intestine	6	19	92
Fat digestibility (%)	85	85	83

[1] Chickens 3 weeks old, 10 birds per isolator, 1 isolator per treatment. The birds were given a semi-synthetic diet with tallow as fat source. Experimental diets were given for 14 days.

[2] Viscosity measured with Brookfield Viscometer DVII+ (shear rate 45 s^{-1}, 40 °C). Upper small intestine: Contents from gizzard to Meckels diverticulum. Lower small intestine: Contents from Meckels diverticulum to ileocecal sphincter

Van der Klis and van Voorst (1993) reported that carboxymethylcellulose significantly increased the average retention time of chyme in the gastrointestinal tract. It is likely that this creates an excellent environment for bacterial activity. The flow of digesta is reduced and the amounts of undigested material in the small intestine is increased. This gives the microflora more time and more substrate to colonise the proximal small intestine. A change in the rheological characteristics of the chyme, and as mentioned previously, in the physicochemical properties of the mucus layer may enhance bacterial adhesion to the mucosal surface. Adhesion between bacteria and epithelial cells is presumed to be important in the pathogenesis of given bacterial diseases. According to Mead (1993) *Enterococci hirae* is a well known example of a microorganism that is capable of colonising duodenal villi and depressing growth. Moreover, most of the soluble NSP sources are fermentable. An increase in bacterial activity in the gastrointestinal tract may cause a systemic effect on the gut secretions and morphology of the small intestine (Sakata, 1987).

Finally, increased bacterial activity may cause an increase in the deconjugation of bile acids and this may impair the return of bile acids to the liver and their subsequent recycling into the bile. As a result, poor digestion of fat may occur by the reduced concentration of bile salts in the digesta and/or

malabsorption by the affected gut wall.

It can be concluded that viscosity may be an important negative property of NSP and that its effects occurs at least partially by the interaction with the gut microflora.

Physiological effects of water holding capacity of insoluble NSPs

Although to a lesser extent than their soluble counterparts, insoluble non-viscous NPSs are able to hold considerable quantities of water (van Soest, 1984; Robertson and Eastwood, 1981). The main effects of the waterholding capacity of insoluble NSPs are the ability to increase the bulk of the chyme and to enhance the digesta passage rate in the small and large intestines. These properties are affected by structure, particle size and fermentability (Robertson, 1988). There is no conclusive evidence that the water-holding properties of non-viscous NSPs impair the digestibility of fat, protein or starch. They might even prove beneficial in situations where there is enhanced bacterial activity in the hindgut. Laxative properties of non-viscous water-holding NSP may diminish the overall bacterial activity in the intestinal tract by decreasing the time available for fermentation in the gut. Moreover, bacteria may adhere to insoluble NSP structures. If it is assumed that the antinutritive effect of viscous NSPs is mainly caused by an increased bacterial activity, then non-soluble NSPs might be capable of partially alleviating the detrimental effects of viscous NSPs. This hypothesis may explain the results obtained by Rogel *et al.* (1987) where an improvement was noted in the starch digestion of low apparent metabolizable energy (AME) wheat after inclusion of oat hulls in the diet.

Physiological effects of binding of ions and surface activity of NSPs

In vitro studies have demonstrated that lignin, pectin and other acidic polysaccharides can adsorb bile acids (Story and Kritchevsky, 1976; Story, 1986). The extent to which the adsorption can increase the excretion of bile salt and fatty acids *in vivo* remains to be established. According to Kritchevsky (1988) the adsorption of bile acids by fibre represents only a small part of the overall effect by which fibre affects lipid and cholesterol absorption. It is possible that the surface properties of NSPs reduce the ileal resorption of bile salts and subsequently enhance their deconjugation by the microflora, thus diminishing the bile acid pool. More information is needed to assess the

importance of this physical property.

Conclusions physiological effects of physico-chemical properties of NSPs

There is sufficient evidence to conclude that solubility and viscosity are important physical properties of NSPs and influence the digestion of fat, protein and starch in broiler chickens. Their effects appear to be mediated by the microflora. The water-holding property of non-viscous NSPs may be beneficial in specific circumstances where there is high bacterial activity in the gut. This is because it decreases the time that the intestinal contents are retained and provides a structure to which bacteria can attach.

The implications of the physicochemical properties of NSPs to practical dietary evaluation

The traditional chemical determination of NSPs

As a result of the absence of the required endogenous enzymes, NSPs are assumed to be essentially indigestible in the upper intestinal tract of birds and monogastric mammals. Because of this, the NSPs are included as part of the 'fibre'. In animal nutrition reference is still made to 'crude fibre' (CF), 'acid detergent fibre' (ADF) and 'neutral detergent fibre' (NDF). Each of these chemical determinations actually measures only part of the fibre as each is based on an aqueous treatment followed by recovery of an insoluble residue. Part of the fibre is lost through dissolution of the NSPs during the aqueous treatment. It is now clear that the soluble NSPs can affect various processes during digestion in broilers. Therefore, determination of NSPs in diets should be based on methods which measure both the soluble and insoluble fractions. In human nutritional science major developments in dietary fibre determinations have occurred over recent years and a variety of assays are available which quantify most of the soluble NSP components. Soluble NSPs are either measured separately or as part of the total dietary fibre. The methods involve enzymatic treatment of the sample in order to remove starch and protein. Quantification is usually achieved by weighing residues (Lee *et al.*, 1992). This has the disadvantage of including some non-NSP components in the residues. Alternatively NSPs

can be determined as their component sugars (following hydrolysis and derivatization) by gas-liquid chromatography or high performance liquid chromatography (Englyst *et al.*, 1992). The disadvantage of this approach is that polyuronates such as pectins are not detected, and can only be quantified after a further colorimetric assay.

It is well established that in barley a soluble NSP, ß-(1→3),(1→4)-D-glucan, is responsible for the poor nutritive value of this cereal for broiler chickens (White *et al.*, 1981, White *et al*, 1983). Soluble ß-(1→3),(1→4)-D-glucan can be determined specifically and conveniently in feedstuff samples using an enzymic-colorimetric method (McCleary and Glennie-Holmes, 1985). Because of the great potential variation in polysaccharide structures it is unlikely that a single NSP assay will ever allow the prediction of nutritive value across all feed ingredients. It is possible that assays specific to particular NPSs will eventually have to be used for the routine quality control of feed ingredients. In the meantime, the use of CF, ADF and NDF should be discouraged and the methods used in dietary fibre analysis of human food adopted.

Published values for the amount of NSPs in a selected range of ingredients used in poultry diets are shown in **Table 3**. Unlike CF, ADF and NDF data there is limited information available on the amounts of NSP (soluble, insoluble and total) in feed ingredients. Two points should be considered when examining the data. Firstly, they represent the results from a number of laboratories where a variety of methods have been used. However, an attempt has been made to present data only from studies where the techniques were comparable. Secondly, the contents of NSPs vary between samples of feedstuffs and this may have profound consequences on their effects in broiler chickens when ingested. For example, Annison (1991) reported that the soluble NSP concentrations in wheat fell within the range 1.26-1.6% DM, values which correlated ($r=-0.91$) with the apparent metabolisable energy contents (AME) of the wheat (range 11.25-13.59 MJ/kg DM). It is clear therefore that studies need to be made examining the concentrations of NSPs in the feed components currently being used in the broiler industry. These determinations should use standardised modern procedures and be applied to as great a number of samples as possible so that the range of values occurring can also be determined.

As has already been stated, the solubility of a NSP depends on many factors. Ideally, any *in vitro* assay to measure the soluble NSPs in a feedstuff should correlate closely with the amount of NSPs that becomes solubilised in the gastrointestinal tract. The methods described above do not meet this important

Table 3 Non-starch polysaccharide content of some ingredients (%DM).

Ingredient	Soluble NSPs	Insoluble NSPs	Total NSPs	Main NSPs and concentration (%DM)
Wheat	2.4[a]	9.0[a]	11.4[a]	Arabinoxylan - 6.05[b]
				β-D-glucan - 0.5[b]
				Cellulose - 2.0[a]
Rye	4.6[a]	8.6[a]	13.2[a]	Arabinoxylan - 8.9[b]
				β-D-glucan - 1.2[b]
				Cellulose - 1.5[a]
Barley	4.5[a]	12.2[a]	16.7[a]	*β-D*-glucan - 7.6[b]
				Arabinoxylan - 3.3[b]
				Cellulose - 3.9[a]
Sorghum				Arabinoxylan - 2.8[b]
				β-D-glucan - 0.1[b]
Maize				Arabinoxylan - 4.2[b]
				β-D-glucan - 0.1[b]
Triticale				Arabinoxylan - 7.0[b]
				β-D-glucan - 0.7[b]
Chick peas				Araban[1] - 4.9[a]
(*Cicer arietinum*)	3.3[c]	7.4[c]	10.7[c]	Cellulose - 2.8[c]
Lupins (*Lupinus*				
angustifolius)	4.0[d]	34.0[d]	38.0[d]	Complex polymer
Peas, late picked				
(*Pisum Sativum*)	2.5[e]	32.2[e]	34.7[e]	Complex polymer
Navy beans				
(*Phaseolus vulgaris*)	5.7[f]	11.7[f]	17.4[f]	Complex polymer
Pinto beans				
(*Phaseolus vulgaris*)	6.3[f]	13.1[f]	19.4[f]	Complex polymer
Soyabean meal				
(*Glycine max*)	13.9[g]	16.4[g]	30.3[g]	Complex polymer
White lupin				
(*Lupinus albus*)				
cotyledons	8.0[g]	20.0[g]	28.0[g]	Complex polymer
Rape seed meal				
(*Brassica campestris*)	11.3[g]	34.8[g]	46.1[g]	Complex polymer

[a] Englyst, 1989; [b] Annison, 1991;[c] Englyst and Cummings, 1988; [d] Cheung, 1991; [e] Haddam and Aman, 1987; [f] Chang et al., 1989; [g] Carré, 1992, adapted from data not corrected for residual proteins. [1] Appears from the levels of the arabinose that arabinan is the main neutral polysaccharide present

criterion. Monro (1993) has proposed a method for determining soluble fibre which has been shown to be more predictive for *in vivo* solubilisation of NSP in rats than the more traditional methods. The procedure differs from others currently in use by extracting soluble fibre before and separately from starch hydrolysis and by using sequential treatments which mimic conditions in the stomach and small intestine.

It should be clear from the earlier discussion, that even distinguishing the NSP component in terms of soluble and insoluble fractions, does not provide a satisfactory explanation for the physiological effects. Other physicochemical properties should be included in assessments of the NSP-components in feedstuffs and their relationship to nutritive values.

Should a description of the physicochemical properties of NSPs be a part of routine feed evaluation?

According to the presently described hypothesis concerning the action of NSPs in birds, in order to be able to predict the nutritive value of an ingredient it will be necessary to determine those physicochemical properties of the NSPs that are closely correlated with the rheology of the digesta and with bacterial activity. Firstly, the viscous properties of NSPs may delay digestion and absorption and may enhance bacterial activity. It is therefore evident that *in vitro* viscosity can provide information for monitoring the nutritional properties of NSPs. Secondly, the amount of soluble NSPs under physiological conditions found in the intestines of chickens may correlate significantly with the amount of fermentable NSPs (Carré, 1993).

Accordingly, solubility of NSPs and *in vitro* viscosity should be included as standard measures in their screening. A general problem is that the methods used for measuring *in vitro* viscosity vary widely. Nevertheless, a determination of the *in vitro* viscosity of a supernatant of a centrifuged suspension prepared under physiological conditions may be appropriate (Bedford and Classen, 1993).

It is assumed that the contribution of the insoluble NSP to viscosity is rather low, and that the addition of insoluble NSPs to the diet does not affect the digestibility of starch, protein and lipids to any great extent. According to our hypothesis, insoluble non-viscous NSPs have physicochemical properties in the intestinal tract that may accelerate the passage rate of digesta and may decrease bacterial activity in the hindgut. The extent to which insoluble NSPs affect digesta passage rate and bacterial activity in broiler chickens is, however, largely

unknown.

In conclusion, An *in vitro* method capable of assessing the effects of dietary NPSs on the nutrition of the chicken would require to provide two pieces of information: the physiological solubilities of the NSPs present in the feedstuff (indicator related to fermentability of the NSPs) and the viscosity of the supernatant of the feedstuff prepared under physiological conditions (indicator related to delayed digestion and absorption and increased bacterial activity). Further research is required to determine precisely which conditions should be used and which parameters should be measured.

Application of the proposed method to account for the physicochemical properties of NSPs

The quality criteria for NSPs proposed above could be immediately to allow a ranking of given feed ingredients for antinutritive properties and this could have application in practical diet formulation. Several workers have related the solubility and *in vitro* viscosity of NSPs to the nutritive value of different varieties and sources of barley and wheat (Campbell *et al.*, 1989; Annison, 1991). However, the magnitude of the effects of the NPSs is dependent on animal and diet factors. Animal-dependent factors are age, health and microbial status, and diet-dependent factors are the amounts of NSPs and the dietary concentrations of other components such as fat, amino acids, vitamins and minerals. Consequently, it is probably too complex to predict exactly the response of the animal to the negative features of NSPs. Lowering the AME value during diet formulation of an ingredient that contains high amounts of soluble NSPs and a high *in vitro* viscosity may not be appropriate as the magnitude of the anti-nutritive effect may depend on the dietary inclusion rate and on the dietary concentrations and compositions of other ingredients. The effect of inclusion rate is clearly demonstrated in **Table 1** from the data of Choct and Annison (1992a) and Fengler and Marquardt (1988). Inclusion rates of 10 and 15 g/kg DM of wheat pentosan did not significantly affect the digestibility of lipids as the animal may have been able to tolerate the low viscosity. The digestive and absorptive capacity of the broilers fed on these diets was evidently sufficient to maintain lipid digestibility at the level of the control birds. However, at inclusion rates of 25 and 30 g/kg DM the pentosan-fraction was capable of exerting its antinutritive properties and subsequently the digestibility of lipids was depressed. It therefore appears that there is a non-linear relationship

between the inclusion rate of soluble viscous NSPs and the digestibility of lipids. In summary, while it is possible to rank the quality of ingredients on the basis of the physicochemical properties of NSPs, it is not appropriate to predict their AME values on this basis and subsequently use them in practical diet formulation.

A way in which knowledge of the physicochemical properties of NSPs may be used in practical diet formulation could be to assume that the broiler has a 'threshold' level below which the antinutritive properties of NSPs are not evident. Ingredients with soluble and viscous NSPs may be included in the diet but their inclusion rate should not exceed this threshold. Some important factors have to be considered. Firstly, the threshold level may be different for the young broiler chicken compared to the chicken at 6 weeks of age as the digestive and fermentative 'capacity' increases with age. Petersen *et al.* (1992) demonstrated that there is a noticeable reduction in *in vivo* viscosity in the foregut as the age of the broiler increases. Secondly, the *in vitro* viscosities of the ingredients may not be additive. Research is needed to derive a method capable of predicting the viscosities of mixed diets from the viscosities of the separate ingredients. The problem of additivity may be circumvented by determining the physicochemical properties of mixed diets. This is not ideal, however, given current least cost feed formulation practices but would serve as a general assessment of formulated diets.

Finally, the determination of soluble NSPs and their *in vitro* viscosities can be applied for determining and describing the effects of various processing techniques on NSP activity in the mixed diets. Depolymerisation of NSPs by enzyme addition to broiler diets lowers the *in vitro* and *in vivo* viscosity and may alter the amount of soluble NSPs. Heat treatment can also affect NSP properties. Irradiation of rye lowers the degree of polymerisation of the pentosan fraction and reduces *in vitro* viscosity (Patel *et al.*, 1980, Campbell *et al.*, 1983b). It was noted by Classen and Bedford (1991) that the solubilities of NSP and their viscosities may be increased by autoclaving, pelleting and extrusion.

The approach currently proposed includes a determination of the physicochemical properties of NSP. Development and validation will be needed before an acceptable technique can be applied.

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Chapter 2
Appendix

Experimental details of data presented in table 2

Animals and diets

Naturally clean broiler-strain eggs (Ross) were incubated, sterilised as described by Yokota et al. (1984), and hatched in a pre-sterilised isolator equipped with thermostatically-controlled supplementary heat sources. Three isolators were used for the experiment and to each isolator 10 germfree broiler chickens were randomly allocated. The isolators, as well as material entering or leaving the isolator were sterilised using peracetic acid (2% w/v) spray. Excreta of the birds were taken every week, and at the end of the experiment, to check for microbial contamination. The germfree status was verified at the end of the experiment.

Three diets were prepared without and with 0.5 and 1.0% carboxymethylcellulose (CMC), a water-soluble, gelling polysaccharide (**Table 1**). Chromium oxide was used as an indigestible marker. The experimental diets were fed to the broiler chickens from 14 to 21 days of age. All diets were sealed in plastic bags and sterilised by irradiation at 20 kRay (Gammaster, Ede, The Netherlands). The control diet was fed to the broiler chickens in the preliminary period.

Collection of samples

All broiler chickens were used for the collection of samples. Excreta was collected from day 15 to 21, pooled per isolator, stored at -20°C and freeze dried for the determination of lipids. At the end of the experiment all birds were killed by an intracardial injection with 2 ml T61® (0.2 g butramide, 0.05 g mebezonicumiodide and 0.005 g tetracainehydrochlorde per ml; Hoechst Veterinär GmbH, München, Germany). The digestive tract between the gizzard and Meckel's diverticulum was removed and considered as duodenum plus jejunum. The ileum was isolated as section between Meckel's diverticulum to the ileocecal junction. The caeca and colon were removed starting from the ileocecal junction. Fresh subsamples of duodenal plus jejunal and ileal contents were immediately centrifugated at 5000xg for 15 min at 4°C. The supernatant was discarded for viscosity measurement.

Analyses

The viscosity of supernatants prepared from the contents of duodenum plus jejunum, ileum and caeca was measured using a cone-plate viscometer (Brookfield digital DV-II+, Brookfield Engineering Labs Inc., Stoughton, UK) that was maintained at 40°C. The values were recorded at a shear rate of 45 s^{-1}.

Table 1 Composition of the experimental diets

Ingredient	g/kg
Wheat starch	350
Maize	200
Soyaprotein isolate	230
Glucose[1]	75
Tallow$_2$	50
Cellulose	30
Monocalcium phosphate	21
Potassium bicarbonate	15
Limestone	12
Vitamin-mineral premix[3]	10
Sodium chloride	3
Dl-methionine	2
Chromium oxide	2
CMC[4]	0, 5 or 10

[1] Glucose was included as part of another study.

[2] Typical fatty acid composition tallow (g/kg): $C_{16:0}$, 215; $C_{16:1}$, 27; $C_{18:0}$, 125; $C_{18:1}$, 366; $C_{18:2}$, 89; $C_{18:3}$, 9.

[3] The vitamin-mineral premix consisted of (mg/kg): thiamin, 2.5; riboflavin, 5.5; *d*-pantothenic acid, 15; niacin amide, 50; pyridoxine, 3; biotin, 0.15; folic acid, 0.75; choline chloride, 1850; cyanocobalamin, 0.015; inositol, 100; para-amino benzoic acid, 2.5; *Dl*-α-tocopheryl acetate, 30; menadione, 5; ascorbic acid, 50; retinyl acetate, 5.2; cholecalciferol, 0.075; $FeSO_4.7H_2O$, 200; $ZnSO_4.H_2O$, 200; $CuSO_4.5H_2O$, 150; MgO, 500; MnO_2,150; KI,5; $CoSO_4.7H_2O$,1; $Na_2SeO_3.5H_2O$, 0.2; ethoxyquin, 100; maize, 6574.1.

[4] CMC of high viscosity (AF2805, Akzo Chemicals, Arnhem, The Netherlands). CMC was exchanged at the expense of wheat starch in diet B and C.

Viscosity was expressed as milli Pascal seconds (mPa.s).

Chromium levels in feed and excreta samples were determined by atomic absorption spectrophotometry (SpectrAA-10, Varian Nederland BV, Houten, The Netherlands). Total lipid contents of feed and excreta were determined by extraction of the samples with hexane following boiling in hydrochloric acid (3M) for 30 min. The apparent lipid digestibility was calculated as follows: $DC_{Lipid} = 1-[(Cr_{feed}/Cr_{excreta})x(Lipid_{excreta}/Lipid_{feed})]$ where, DC_{Lipid} = apparent digestibility of lipids, $Cr_{feed,excreta}$ = chromium content in feed or excreta and $Lipid_{feed,excreta}$ = lipid content in feed or digesta.

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Chapter 3

Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens

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Submitted

Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens

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Abstract

Water-soluble carboxymethylcellulose preparations of low (LCMC) or high viscosity (HCMC) were fed to broiler chickens. HCMC versus LCMC reduced weight gain and raised feed and water intake. After feeding the HCMC diet, viscosity of the supernatant of small intestinal contents was significantly raised. HCMC raised the group mean ATP concentration in the digesta of duodenum plus jejunum, indicating that bacterial activity was increased. HCMC feeding depressed apparent faecal digestibility of lipids and also apparent ileal digestibility of starch and nitrogen. HCMC in the diet tended to reduce plasma triglyceride levels. After HCMC consumption the weights of the small intestine and colon, without or with contents, were elevated. The data indicate that a high viscosity of digesta in broiler chickens is associated with a reduced macronutrient digestion and impaired growth performance. Since the CMC preparations were found to be non-fermentable by fresh faeces, it is suggested that HCMC reduces macronutrient digestion, through raising the viscosity of small intestinal contents, which is followed by enhanced bacterial fermentation due to accumulation of undigested material.

Introduction

The water-soluble, non-starch polysaccharides (NSPs) occurring in rye, wheat and barley are held responsible for the reduction of growth performance and digestibility of lipids, protein and starch in broiler chickens fed these feedstuffs (Choct and Annison, 1992a, Fengler and Marquardt, 1988, White *et al.*, 1981). On the basis of a literature review Annison (1993) proposed that dietary soluble NSPs inhibit nutrient absorption in broiler chickens both by raising the viscosity of the digesta and by enhancing bacterial fermentation. Gel forming plant NSPs

generally are readily fermentable (Roberfroid, 1993) and thus it is difficult to assess separately the anti-nutritive effects of viscosity and fermentability. The effect of viscosity *per se* can be studied by the use of non-fermentable carboxymethylcellulose (CMC) preparations with different degrees of viscosity. Thus, in the present study water-soluble CMCs with varying polymer length were fed to broiler chickens to investigate their impact on growth performance and on digestibility of macronutrients.

Materials and methods

Animals and diets

One day old, female broiler chickens (Ross, Cobroed, Lievelde, The Netherlands) were housed in wire-bottomed, suspended cages, exposed to constant light and given free access to feed and water throughout. During the first three weeks of age all birds were fed the pelleted diet with CMC of low viscosity (**Table 1**), except that the cellulose component was of another type (cellulose type BWW40, Rettenmaier und Söhne, Ellwangen/Jagst, Germany).
Then, 8 groups of 10 birds each were composed so that the groups had similar body weight distributions. Four groups received the diet with cellulose of low viscosity (LCMC diet, Table 1) and the other four groups were fed the diet with cellulose of high viscosity (HCMC diet, Table 1). The birds were randomly allocated to pens so that there were four pens per dietary treatment. The experimental, pelleted diets were fed to the broiler chickens from 21 to 35 days of age. Body weights were determined at the beginning and at the end of the experiment. Feed and water intake were recorded.

Collection of samples

During the last 3 days of the experimental period the excreta in each cage were collected daily and stored at -20°C. The excreta were pooled per cage, freeze dried and milled using a 1 mm sieve. At the end of the experiment all birds were killed by intravenous injection with 2 ml of T61 containing 0.4 mg butramide, 0.1 mg mebezonicumiodide and 0.01 mg tetracainehydrochloride (Hoechst Veterinär, GmbH, München, Germany). Before injection, blood samples were taken by heart puncture. Plasma was collected by low-speed centrifugation and stored at -20°C until lipid analyses. The digestive tract

Table 1 Composition of the experimental diets

Ingredient	g/kg
Corn	340
Corn starch	300
Soybean protein isolate	200
Animal fat	50
Ground limestone	10
Monocalcium phosphate	14
Potassium bicarbonate	10
DL-methionine	2
Vitamin-mineral premix[1)]	12.5
LCMC[2)] or HCMC[3)]	10
Cellulose[4)]	50
Chromium trioxide	1.5

[1)] The vitamin/mineral premix supplied per kg diet (mg):
thiamin, 1; riboflavin, 6; calcium pantothenate, 12; niacin amide, 40; pyridoxine, 2; cyanocobalamin, 0.0225; choline chloride, 369; folic acid, 1; biotin, 0.065; retinyl acetate, 25; cholecalciferol, 0.05; DL-α-tocopheryl acetate, 32.5; menadione, 1.8; ascorbic acid, 20; MgO, 995; MnO_2, 134; $ZnSO_4H_2O$, 155; $FeSO_4H_2O$, 233; $CuSO_45H_2O$, 50; $Na_2SeO_35H_2O$, 0.6; $CoSO_47H_2O$, 0.6; KI, 4; ethoxyquin, 100.
[2)] CMC of low viscosity (AF1985, Akzo Chemicals, Arnhem, The Netherlands)
[3)] CMC of high viscosity (AF2805, Akzo Chemicals, Arnhem, The Netherlands)
[4)] Cellulose type BC1000 (Rettenmaier und Söhne, Ellwangen/Jagst, Germany)

between the gizzard and Meckel's diverticulum was removed to obtain the duodenum plus jejunum. The ileum was isolated as the intestine between Meckel's diverticulum and the ileocecal junction. The caeca and colon were removed as intestine distal to the ileocecal junction. Intestinal contents were collected by gently finger stripping the intestinal segments. The weights of pancreas, liver and intestinal segments with or without contents were recorded. After collection, the intestinal contents were immediately placed on ice and pooled per segment per cage. Portions of the contents were stored at -80°C for ATP analysis. Portions of the ileal contents pools were stored at -20°C and subsequently freeze dried. Fresh samples of pooled duodenal plus jejunal and ileal contents were immediately centrifuged at 5,000xg for 15 min at 4°C in a MSE mistral 3000i centrifuge (MSE, Leicester, UK) using a Windshield rotor (type 43124-708 BS 4402, Beun de Ronde BV, Abcoude, The Netherlands). The supernatant was collected for viscosity measurement.

Analyses and measurements

Suspensions of the two CMC types (1% w/w) and of the experimental diets (10% w/w), that had been milled at a 1 mm sieve, were prepared in distilled water and incubated for 30 min at 38°C for *in vitro* viscosity measurements. The diet suspensions were centrifuged at 6000 x g for 15 min and the supernatant was isolated for the viscosity measurements. The viscosity of the CMC suspensions and the supernatants of diet suspensions was measured at a shear rate of 0.0623 s^{-1} and a temperature of 38°C using a Bohlin VOR Rheometer (Bohlin, Reologi, Mühlacker, Germany).

For the determination of the *in vitro* fermentability of the CMC preparations, a 32 % (w/w) faecal slurry was made by using fresh human faeces and sodium phosphate buffer (0.1 M, pH 6.5). The slurry was kept under a continuous flow of nitrogen gas. Five ml of the slurry was mixed with 5 ml of a sodium phosphate buffer (0.1 M) without addition or containing either 2 % (w/v) citrus pectin, LCMC or HCMC. The citrus pectin served as a positive control and the buffer without addition as a negative control. For each condition five plastic tubes were used to determine the fermentation products after 0, 1, 3, 6 and 24 h. The incubation tubes were placed in a water bath at 37°C. At each time interval one tube was removed, centrifuged during 10 minutes at 4260 x g and 4°C, and the supernatant collected and stored at -20°C. The amounts of acetic, propionic, butyric and valeric acid in the supernatant were determined by the method of Tangerman *et al.* (1983).

The viscosity of supernatants prepared from the contents of duodenum plus jejunum and ileum was measured using a cone-plate viscometer (Brookfield digital DV-II+, Brookfield Engineering Labs Inc., Stoughton, United Kingdom) that was maintained at 40°C. The values were recorded at a shear rate of 45 s^{-1}. Viscosity was expressed as milli Pascal seconds (mPa.s). The pH of intestinal contents was determined immediately after collection using a pH meter (PHM 82 Standard pH meter, Radiometer Nederland, Zoetermeer, The Netherlands). Triglyceride and cholesterol levels plasma were determined with the use of commercial test combinations (CHOD-PAP and GPO kits, Boehringer-Mannheim GmbH, Mannheim, Germany). Chromium in 1 g samples of feed and freeze dried chyme and excreta was determined by atomic absorption spectrophotometry (SpectrAA-10, Varian Nederland BV, Houten, The Netherlands) after the samples had been ashed at 550°C and oxidized by the addition of 6 ml of potassium bromate (3 %, w/v) and 3 ml of a solution containing magnesium sulphate (0.067 %, w/v) and orthophosphoric acid (82.45 %, w/v). Total lipid

contents of feed and excreta were determined by extraction of the samples with hexane in a Sohxlet tube after they had been boiled in hydrochloric acid (3M) for 30 min. The determination of nitrogen in feed, ileal digesta and excreta was carried out with the Kjeldahl method. Faecal nitrogen in the excreta was calculated as total nitrogen minus nitrogen in uric acid. Uric acid was analyzed by the method of Terpstra and De Hart (1974). Starch in feed and ileal digesta was analyzed according to the NEN 3574-procedure (NNI, 1974). The concentration of ATP in chyme was estimated by the luciferin-luciferase (EC 1.13 12.7) method as described by Bach-Knudsen *et al.* (1991) with the use of ATP monitoring kits (Microbial Biomass Testkit Lumac 93221-1, ATP Standard Lumac 9263-8) and a luminometer (Lumac Biocounter M1500, Perstorp Analytical, Oud Beijerland, The Netherlands).

Statistical analyses

The level of statistical significance was pre-set at $P<0.05$. The two-tailed Student's *t*-test was used to identify statistically significant differences between HCMC and LCMC feeding.

Results

The HCMC preparation was found to have a 60% higher viscosity than the LCMC preparation (**Table 2**). The supernatant of the diet with HCMC had a 70% higher viscosity than that of the diet with LCMC. The addition of pectin to fresh faeces raised the production of short-chain fatty acids, but the addition of either LCMC or HCMC did not have a stimulatory effect (**Figure 1**).

Feeding the diet with HCMC instead of LCMC raised the viscosity of the supernatant of chyme from the duodenum plus jejunum and from the ileum (**Table 3**).

The pH of the intestinal contents was not affected by the type of CMC. HCMC feeding produced a rise in ATP levels, but the difference only reached borderline statistical significance for the chyme of duodenum plus jejunum.

The feeding of HCMC raised feed and water intake, but reduced weight gain (**Table 4**). Thus, the feed to gain ratio was significantly elevated after feeding the HCMC diet when compared with the LCMC diet.

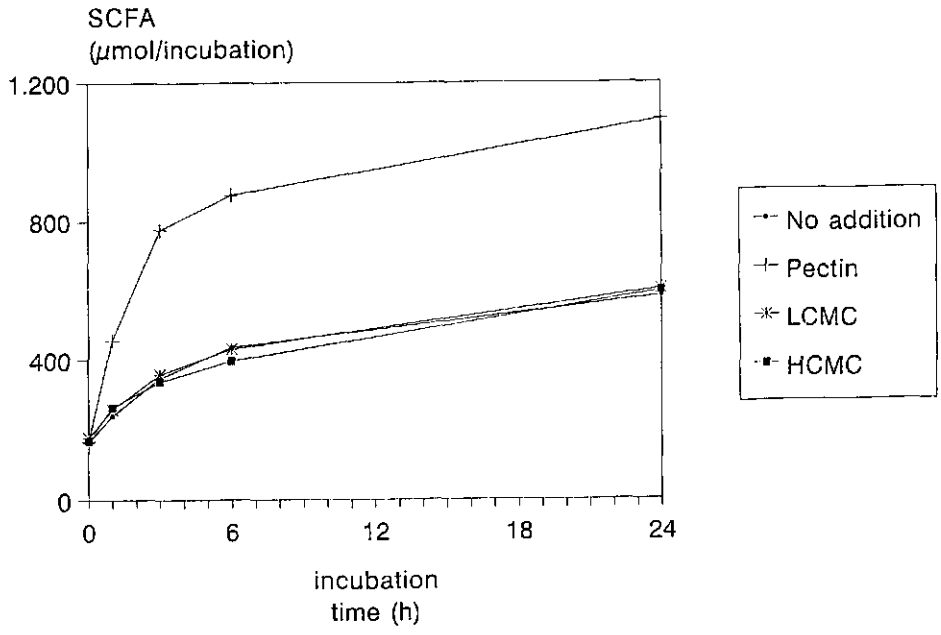
Table 2 *In vitro* viscosity of the LCMC and HCMC preparations and the experimental diets

	Viscosity (mPa.s)
LCMC preparation	130[1]
HCMC preparation	207
LCMC diet	2.7[2]
HCMC diet	4.6

[1] Viscosity of a 1% (w/w) suspension.

[2] Viscosity of the supernatant of a 10% (w/w) suspension.

Figure 1: *In vitro* fermentability of LCMC and HCMC



Time course of *in vitro* fermentation in fresh faeces incubated with buffer alone or with buffer containing identical amounts of either pectin, LCMC or HCMC. Fermentation was monitored by the formation of short chain fatty acids (SCFA).

Table 3 Viscosity of supernatant, pH and ATP contents of digesta in broiler chickens fed either the LCMC or HCMC diet

	Dietary group		
	LCMC	HCMC	Level of significance
Viscosity of supernatant, mPa.s			
. Duodenum plus jejunum	7.90[1)] ± 1.75	17.38 ± 1.05	$P=0.001$
. Ileum	17.05 ± 2.06	28.65 ± 7.59	$P=0.062$
pH			
. Duodenum plus jejunum	5.24 ± 0.53	5.40 ± 0.21	$P>0.500$
. Ileum	5.38 ± 0.19	5.21 ± 0.22	$P=0.369$
. Caeca	6.38 ± 0.10	6.22 ± 0.16	$P=0.144$
ATP, mmol/l			
. Duodenum plus jejunum	1.87 ± 0.52	2.65 ± 0.46	$P=0.058$
. Ileum	4.33 ± 1.33	4.59 ± 1.27	$P>0.500$
. Caeca	21.50 ± 6.74	27.47 ± 3.23	$P=0.213$

Results are means ± SD of four replicates per dietary group.

Table 4 Weight gain, feed intake, water intake and feed conversion ratio of broiler chickens fed either the LCMC or HCMC diet from 21 to 35 days of age.

	Dietary Group		
	LCMC	HCMC	Level of significance
Weight gain, g	583 ± 16	480 ± 15	$P=0.004$
Feed intake, g	1059 ± 40	1115 ± 34	$P=0.096$
Water intake, g	1998 ± 166	2475 ± 147	$P=0.009$
Water : feed ratio, g/g	1.885 ± 0.102	2.218 ± 0.092	$P=0.014$
Feed : gain ratio, g/g	1.815 ± 0.029	2.324 ± 0.063	$P=0.001$

Results are means ± SD of four replicates per dietary group

The full and empty weights of small intestine and colon, expressed relative to body weight, were significantly raised by HCMC in the diet (**Table 5**). Moreover, HCMC consumption produced an increase in length of the small intestine, caeca and colon. The full weight of the caeca was not significantly influenced by the type of CMC in the diet, but the empty weight was significantly elevated. Pancreas and liver weight were not different for broilers fed the diets with either HCMC or LCMC.

Feeding HCMC instead of LCMC reduced the apparent ileal digestibility of starch and nitrogen and the apparent faecal digestibility of nitrogen and lipids (**Table 6**).

Ingestion of HCMC versus LCMC reduced the group mean plasma concentrations of triglycerides and cholesterol, the effect on triglycerides approaching statistical significance (**Table 7**).

Discussion

The objective of this study was to determine whether a raised viscosity of intestinal contents by itself may cause depressed growth performance and reduced macronutrient digestibility in broiler chickens. To meet the objective, broilers were given diets with either HCMC or LCMC. The two types of CMC were verified to be non-fermentable by faecal bacteria, but to show different degrees of viscosity after feeding to the broiler chickens. HCMC produced a markedly higher viscosity of small intestinal contents than did the ingestion of LCMC.

When compared with LCMC, HCMC had an anti-nutritive effect in broiler chickens. HCMC feeding depressed growth performance, raised water intake and inhibited macronutrient digestion. Similar results have been obtained earlier in broiler chickens after the feeding of isolated viscous NSPs of plant origin such as water-soluble wheat pentosans (Choct and Annison, 1992a), water-soluble rye pentosans (Antoniou & Marquardt, 1981, Fengler and Marquardt, 1988) and barley ß-glucans (White *et al.*, 1981). Our data indicate that a raised viscosity *per se* of intestinal contents may be associated with the anti-nutritive effects. Enhanced fermentation after the ingestion of gel forming plant NSPs could contribute to the anti-nutritive effects as suggested by Annison (1993).

HCMC versus LCMC feeding depressed the apparent digestibility of starch,

Table 5 Weights of the intestinal segments, pancreas and liver in broiler chickens fed either the LCMC or HCMC diet

	Dietary group		
	LCMC	HCMC	Level of significance
Small intestine			
. Full weight, g/100 g b.w.[1)]	5.10 ± 0.49	6.83 ± 0.62	$P=0.026$
. Empty weight, g/100 g b.w.	3.28 ± 0.23	4.52 ± 0.30	$P=0.009$
. Length, cm/100 g b.w.	10.78 ± 0.23	13.72 ± 0.86	$P=0.008$
Caeca			
. Full weight, g/100 g b.w.	0.74 ± 0.09	0.69 ± 0.04	$P=0.378$
. Empty weight, g/100 g b.w.	0.42 ± 0.02	0.49 ± 0.03	$P=0.009$
. Length, cm/100 g b.w.	2.16 ± 0.08	2.52 ± 0.16	$P=0.030$
Colon			
. Full weight, g/100 g b.w.	0.21 ± 0.04	0.27 ± 0.01	$P=0.023$
. Empty weight, g/100 g b.w.	0.17 ± 0.03	0.24 ± 0.01	$P=0.009$
. Length, cm/100 g b.w.	0.51 ± 0.04	0.61 ± 0.07	$P=0.027$
Total intestinal tract			
. Full weight, g/100 g b.w.	6.04 ± 0.44	7.79 ± 0.67	$P=0.027$
. Empty weight, g/100 g b.w.	3.87 ± 0.24	5.26 ± 0.33	$P=0.007$
. Length, cm/100 g b.w.	13.44 ± 0.20	16.84 ± 1.04	$P=0.009$
Pancreas, g/100 g b.w.	0.24 ± 0.02	0.25 ± 0.01	$P=0.107$
Liver, g/100 g b.w.	3.40 ± 0.42	3.48 ± 0.20	$P>0.500$

Results are means ± SD of four replicates per dietary group.

[1)] B.w. = body weight.

Table 6 Digestibility of nitrogen, starch and lipids in broiler chickens fed either the LCMC or HCMC diet

	Dietary group		
	LCMC	HCMC	Level of significance
Nitrogen			
. Ileal digestibility[1], %	71.33 ± 3.58	57.31 ± 11.93	$P=0.162$
. Faecal digestibility[2], %	81.63 ± 2.34	65.82 ± 2.53	$P=0.004$
Starch			
. Ileal digestibility[1], %	95.38 ± 1.58	87.34 ± 3.64	$P=0.026$
Lipids			
. Faecal digestibility[1], %	62.14 ± 9.78	34.34 ± 6.54	$P=0.002$

Results are means ± SD of four replicates per dietary group

[1] Calculated as: $DC_{feed} = (1 - [(M_{feed}/M_{e,c}) \times (C_{e,c}/C_{feed})]) \times 100$ where DC_{feed} = digestibility of a nutrient in the feed; M_{feed} = marker (chromium) concentration in feed; $M_{e,c}$ = marker concentration in excreta (e) or chyme (c); C_{feed} = concentration of nutrient in feed; $C_{e,c}$ = concentration of nutrient in excreta (e) or chyme (c).

[2] Corrected for the concentration of uric acid in the excreta (Terpstra and De Hart, 1974).

Table 7 Plasma triglyceride and cholesterol levels in broiler chickens fed either the LCMC or HCMC diet

	Dietary group		
	LCMC	HCMC	Level of significance
Triglycerides, mmol/l	0.58 ± 0.11	0.44 ± 0.10	$P=0.077$
Cholesterol, mmol/l	2.93 ± 0.18	2.45 ± 0.42	$P=0.183$

Results are means ± SD of four replicates per dietary group.

nitrogen and lipids. The mechanism by which an increase in digesta viscosity may affect macronutrient digestion is by no means clear. Possible processes involved are a reduction of the diffusion rate of digestive enzymes and less mixing of chyme (Edwards *et al.*, 1988, Isaksson *et al.*, 1982). In the contents of duodenum plus jejunum from chickens fed HCMC there was an increase in ATP concentration, pointing at enhanced microbial activity. Since HCMC itself is not fermented, the rise in microbial activity probably is due to accumulation of non-digested material. In this light, the primary effect of HCMC is elevating the viscosity of intestinal digesta, causing depressed macronutrient digestion followed by enhanced bacterial fermentation. In addition, an increase in viscosity of intestinal digesta may raise its retention time (van der Klis *et al.*, 1993) which would also stimulate fermentation. The material sustaining fermentation at least consists of carbohydrates and/or protein rather than lipids, because the latter compounds will not serve as bacterial substrate.

Lipid digestibility was more depressed by HCMC than was the digestibility of protein or starch. A similar pattern was noted in birds after feeding diets containing isolated water-soluble pentosans (Choct and Annison, 1992a and 1992b, Fengler and Marguardt, 1988). In young birds the low bile acid concentration in the digesta may limit lipid absorption (Edwards, 1962, Gomez & Polin, 1976, Iñarrea *et al.*, 1989, Polin *et al.*, 1980). Various authors have suggested that the low lipid digestibility in broiler chickens fed diets with a high content of gelling NSPs may be due to bacterial overgrowth in the small intestine and subsequent excessive deconjugation of bile acids (Cole and Boyd, 1967, Huhtanen and Pensack, 1965, Salih *et al.*, 1991). A marked increase in microbial cholyltauryl hydrolase activity has been observed in chicks given rye (Feighner and Dashkevicz, 1988). This observation could imply that a high viscosity of intestinal digesta may not be the primary cause for the decrease in lipid digestion seen after HCMC feeding. Addition of sodium taurocholate to a rye-based diet improved apparent lipid digestibility in broiler chickens (Campbell *et al.*, 1983). In HCMC-fed birds there might be a decreased bile acid concentration in the digesta, which could then not only explain the low lipid digestibility, but also the low plasma triglyceride and cholesterol concentrations as secondary features. Gallaher *et al.* (1993) found a reduced plasma cholesterol level in hamsters fed diets containing hydroxypropyl methylcellulose with varying viscosity.

The observed hypertrophic effect of HCMC on the small intestine, caeca and colon could be induced by the increase in microbial fermentation that occurs secondary to the decrease in macronutrient digestion. Volatile fatty acids and

polyamines produced by gut bacteria have direct and systemic hypertrophic effects and stimulatory effects on the proliferation rate and secretory activity of intestinal mucosa (Furuse *et al.*, 1991, Osborne and Seidel, 1989, Sakata, 1987, Seidel *et al.*, 1985). As a result there would be more loss of endogenous nitrogen, leading to a reduction of apparent nitrogen digestibility as was indeed seen in HCMC-fed birds. Angkanaporn *et al.* (1994) demonstrated that water-soluble pentosans in the diet significantly raised the endogenous nitrogen losses in broiler birds. Larssen *et al.* (1994) found an increase in endogenous nitrogen loss when highly viscous CMC was added to the diet of rats, while ileal true nitrogen digestibility was not affected. Thus, in those rats viscous CMC did not influence nitrogen digestibility, at least not at the level of the ileum. If and when the situation in rats extends to our broiler chickens, then HCMC does not primarily interfere with protein digestion followed by enhanced fermentation. Our reasoning above would lead to the idea that the primary effect of HCMC involves depression of carbohydrate digestion. The increase in microbial growth could indirectly inhibit lipid digestion and lower apparent nitrogen digestibility.

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Chapter 4

The inhibitory effect of a highly viscous carboxymethylcellulose on dietary fat digestibility in the growing chicken is dependent on the type of fat

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Submitted

The inhibitory effect of a highly viscous carboxymethylcellulose on dietary fat digestibility in the growing chicken is dependent on the type of fat

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Abstract

The aim of the study was to determine whether there is an interaction between type of fat and lipid digestibility, for diets which induce an increased digesta viscosity. Pairs of semi-synthetic diets were formulated which contained either tallow, soyabean oil or coconut oil as the main source of fat. To one diet of each pair, carboxymethylcelullose (CMC) was added. CMC is a soluble, viscous non-fermentable fibre. The fat fraction of the tallow diet comprised mainly $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids, while for the soyabean oil diet $C_{18:1}$ and $C_{18:2}$ fatty acids predominated and for the coconut oil diet $C_{12:0}$ and $C_{14:0}$ fatty acids were predominant. The diets were fed to broiler chickens (7 to 21 days of age) in a conventional digestibility study using chromium oxide as an indigestible marker.

CMC significantly raised the viscosity of the liquid phase of the small intestinal digesta and increased the ratio of liquid to solids. CMC depressed apparent faecal lipid digestibility for chickens fed the tallow diet (76.1 vs 66.0%) but not for the birds given the soyabean oil or coconut oil diets. The apparent digestibility of $C_{16:0}$ was significantly depressed in the birds fed the tallow diet (62.7 vs 52.3%) and the digestibility of $C_{18:0}$ tended to be lower. There was no significant effect of CMC on the digestibility of $C_{18:1}$ and $C_{18:2}$ in the soyabean oil diet nor on the digestibility of $C_{12:0}$ or $C_{14:0}$ in the coconut oil diet. The effect of CMC induced digesta viscosity on lipid digestibility in chickens is dependent on the lipid source and its fatty acid composition.

Introduction

The ingestion of soluble, viscous and readily fermentable non-starch polysaccharides (NSPs) present in rye, wheat and barley, reduces growth performance and macronutrient digestibility in broiler chickens (Choct & Annison, 1990 and 1992a; Fengler & Marquardt, 1988; White *et al.*, 1981). The mode of action of the NSPs is poorly understood, but the observed increase in the viscosity of digesta and enhanced bacterial fermentation after feeding NSPs may be involved (Annison, 1993; Bedford & Classen, 1992; Smits & Annison, 1996). It is difficult to assess the relative importance of viscosity versus fermentability as determinants of the antinutritive effect of the NSPs. The primary effect of viscosity can be studied, however, by using non-fermentable high viscosity carboxymethylcellulose (CMC). On the basis of a study with broiler chickens that were fed CMC with either low or high viscosity, we have proposed the following cascade of events (Smits *et al.*, 1996). Feeding highly viscous CMC raises the viscosity of digesta which inhibits starch and protein digestion and glucose and amino acid absorption in the proximal small intestine. The accumulation of substrate stimulates bacterial fermentation which in turn enhances the secretory activity of intestinal mucosa, leading to an increased loss of endogenous nitrogen and a decrease in apparent nitrogen digestibility. The increase in bacterial activity also enhances bacterial deconjugation of bile acids so that micelle formation is depressed and fat digestibility is lowered.

If the feeding of highly viscous CMC indeed interferes with micelle formation, then its effect on fat digestibility should be dependent on the type of dietary fat. Pancreatic lipase rapidly releases fatty acids of medium chain length ($C_{8:0}$ to $C_{14:0}$) that can, due to their relatively high water solubility, be taken up by enterocytes without prior incorporation into micelles (Sallee & Dietschy, 1973; Westergaard & Dietschy, 1976). The absorption of long chain saturated ($C_{16:0}$, $C_{18:0}$) and polyunsaturated fatty acids ($C_{18:2}$) from dietary triacylglycerols is also dependent on lipolysis by lipase, but the saturated fatty acids are less rapidly incorporated into micelles than the polyunsaturated fatty acids (Bézard and Bugaut, 1986; Polin *et al.*, 1980; Young *et al.*, 1963). Thus, it can be hypothesized that the feeding of CMC with high viscosity has a greater inhibitory effect on fat digestibility when the ration contains tallow, which is rich in $C_{16:0}$ and $C_{18:0}$, than when it contains coconut oil which is rich in $C_{12:0}$ and $C_{14:0}$. The proposed inhibitory effect is expected to be intermediate with soyabean oil that is

relatively rich in $C_{18:2}$. The present study with broiler chickens aimed to test this hypothesis.

Materials and methods

Animals and diets

Six semi-purified test diets were formulated (**Table 1**). Pairs of diets contained either tallow, soyabean oil or coconut oil as the main source of dietary fat. A highly viscous carboxymethylcellulose (CMC AF 2905, Akzo Chemicals, Arnhem, The Netherlands) was added (10 g/kg) to one diet of each pair. The total fat and selected fatty acid contents of the diets are shown in **Table 2**. The CMC was added at the expense of a portion of the cellulose component (BWW40, Rettenmaier und Söhne, Ellwangen/Jagst, Germany). All diets contained chromium oxide as an indigestible marker. One-day-old, female Ross broiler birds (n = 108) were housed in suspended wire-bottomed cages placed in three batteries. The temperature was controlled at 30°C at day 1 and gradually reduced to 25°C at the end of the trial. The birds were given a 24-hour light schedule. The animals were allowed to adapt to the environment for a period of two days during which they received a starter diet and water *ad libitum*. The starter diet contained a mixture of 2.6% of each test fat and 327 g/kg maize starch, and was otherwise identical to the CMC-free, experimental diets. After two days, three birds were randomly assigned to each cage and treatments were randomly allocated to cages such that there were six cages (18 birds) per dietary treatment. The starter diet was fed for a further 6 days. From day 8 to day 22 the broiler chickens were given the experimental diets (Table 1) *ad libitum*. The chickens had free access to water and daily feed intakes were recorded.

Collection of samples

During the last four days of the experiment, total excreta were collected and were stored at -20°C. At the end of the experiment, the birds were killed by an intracardial injection of sodium pentobarbitone. The digestive tract from the gizzard to the colon was removed and the intestinal contents of each bird from Meckel's diverticulum to the ileocecal junction were collected by gentle manual expression. Digesta for the three birds from the same cage were pooled and were kept at 4°C until determination of viscosity, which was made on the same day of digesta collection.

Table 1 Composition of the experimental diets (g/kg)

	Tallow	Soya-bean oil	Coconut oil	Tallow	Soya-bean oil	Coconut oil
CMC	-	-	-	+	+	+
Tallow	80	-	-	80	-	-
Soyabean oil	-	80	-	-	80	-
Coconut fat	-	-	80	-	-	80
Cellulose	40	40	40	30	30	30
CMC	-	-	-	10	10	10
Constant components[1]	880	880	880	880	880	880

[1] The constant components consisted of (g): maize, 300; corn starch, 325; isolated soya protein, 200; ground limestone, 13; dicalcium phosphate, 21; iodised salt, 3; potassium bicarbonate, 10; DI-methionine, 3; vitamins and minerals, 3; chromium oxide 2. The vitamin and mineral mixture supplied per kg of feed (mg/kg): retinyl acetate, 22; cholecalciferol, 4.8; DL-*α*-tocopherolacetate, 60; menadione, 4; thiamin, 3; riboflavin, 12; nicotinic acid, 35; folic acid, 5.2; pyridoxine-HCl, 10; cyanocobalamin, 0.017; choline chloride, 638; d-pantothenic acid, 2.8; biotin, 0.2; MgO, 829; MnO_2, 198; $ZnSO_4.H_2O$, 166; $FeSO_4.7H_2O$, 83; $CuSO_4.5H_2O$, 38; $CaI_2.6H_2O$, 1.6; $Na_2MoO_4.2H_2O$, 1.0; $Na_2SeO_3.5H_2O$, 0.75; $CoSO_4.7H_2O$, 0.50; limestone, 825; ethoxyquin, 50.

Analyses and measurements

For viscosity determination, the digesta samples were centrifuged for 5 min at 15.000xg. The weights of supernatant and solids were measured for the determination of the ratio liquid/solid. Rheological measurements were carried out using a Bohlin VOR Rheometer (Bohlin Reologi, Mühlacker, Germany). The viscosity of approximately 0.5 ml of supernatant was determined using a Bohlin cone/plate (2.5/30) and a 0.307 g/cm torsion bar at 38°C. The viscosity at a shear rate of 320 s^{-1} was taken as the viscosity value of interest.

Excreta were thawed, mixed, freeze dried and ground (1 mm sieve). Chromium was determined in duplicate following the method of Costigan & Ellis (1987) after the samples had been digested as described by Williams *et al.* (1962). Total lipid contents of the diets and excreta were determined gravimetrically by extraction of the samples with hexane using a Sohxlet apparatus, following boiling in hydrochloric acid (3 M) for 30 minutes. Fatty acid analysis was performed essentially according to the procedures NEN 5410a and 5410b (NNI, 1996). Methyl esters of free fatty acids were separated using a gaschromatographer (GC MEGA 8160-OS, Fisons Instruments, Breda, The

Table 2 Total lipid content and selected fatty acid concentrations (g/kg) for the experimental diets[1]

	Tallow	Soyabean oil	Coconut oil
Total lipid	109	108	108
$C_{12:0}$	<1	<1	40.2
$C_{14:0}$	2.4	<1	14.8
$C_{16:0}$	22.4	13.4	10.7
$C_{18:0}$	19.4	4.1	8.5
$C_{18:1}$	28.7	20.6	4.9
$C_{18:2}$	10.6	38.5	11.4

[1] Mean values for diets with and without CMC.

Netherlands) equipped with on-column injection and flame ionisation detection (FID) over a fused silica capillary column (DB-23, J+W Scientific, Folsom, USA) by using temperature programming. Helium was used as carrier gas. Heptadecanoic acid ($C_{17:0}$) was added to each sample as an internal standard. The apparent digestibility of total lipid and selected fatty acids was calculated as: $DC_{diet} = (1-[(Cr_{diet}/Cr_{excr})x(L,F_{excr}/L,F_{diet})])x100$ where DC_{diet} = apparent digestibility of either total lipid (L) or fatty acids (F) in the diet; Cr_{diet} = concentration of chromium in the diet; Cr_{excr} = concentration of chromium in the excreta; L,F_{excr} = concentration of either total lipid or fatty acids in the excreta; L,F_{diet} =concentration of either total lipid or fatty acids in the diet.

Statistical analysis

The data were subjected to an analysis of variance with fat type (tallow, soyabean oil or coconut fat) and amount of CMC (0 or 10 g/kg) as dietary variables. The generalised model for the analysis of variance was $Y_{ijk} = \mu + B_i + T_j + e_{ijk}$, where Y_{ij} = observation, μ = mean, B_i = Block, T_j = treatment and e_{ijk} = residual variation. Treatment means were compared by a least significant difference (LSD, Snedecor & Cochran, 1967). Diet effects and differences between treatment means were considered to be significant at $P \leq 0.05$.

Table 3 Viscosity of the liquid phase of small intestinal digesta and the ratio liquid:solid of digesta for broiler chickens fed the experimental diets.

	Tallow		Soyabean oil		Coconut oil			
CMC	-	+	-	+	-	+	SED[1]	Source of variation[2]
Viscosity (mPa.s)	6.1[a,3]	71.8[b]	5.1[a]	45.9[b]	3.8[a]	64.3[b]	13.8	CMC, Fat type x CMC
Liquid:solid ratio (w:w)	0.088[a]	0.215[b]	0.097[a]	0.252[b]	0.089[a]	0.320[b]	0.047	CMC

[1] SED = Standard Error of Difference.
[2] $P<0.05$.
[3] Means in the same row with different superscripts differ significantly ($P<0.05$).

Results

There were no significant differences between the treatments for feed intake of the animals. The average total feed intake over the entire feeding period of 14 days was 488 g (SD ± 20.1). CMC significantly increased the viscosity of the liquid phase of the ileal digesta (Table 3) and CMC also increased the ratio of liquid to solids in the digesta. There was a significant Fat x CMC interaction with the increase in digesta viscosity being less for birds fed the soyabean oil diet in comparison to the other diets.

The apparent digestibility of total lipid was significantly lower for the diets containing tallow when compared to those containing either soyabean oil or coconut oil. This was due to the low digestibility of the $C_{16:0}$ and $C_{18:0}$ fatty acids. When either soyabean oil or coconut oil was fed, the digestibility of $C_{18:0}$ was significantly higher than when tallow was fed. The birds fed the soyabean oil diet had the highest digestibility of $C_{16:0}$ and $C_{18:0}$. $C_{18:2}$ was digested more efficiently when derived from soyabean oil rather than from tallow or coconut oil. The digestibility of $C_{18:1}$ and $C_{18:2}$ was significantly lower in the broiler chickens receiving the coconut oil diet.

CMC significantly depressed the apparent digestibility of total lipid for broiler chickens fed tallow but this was not observed for their counterparts fed either the soyabean oil or coconut oil containing diet. The digestibility of $C_{16:0}$ was significantly reduced by CMC addition for the birds receiving the tallow diet. In birds given the soyabean oil diet there was a significant effect on the digestibility

of $C_{18:0}$. CMC depressed the digestibility of $C_{18:1}$ from either tallow or soybean oil, but not from coconut oil. The digestibility of $C_{12:0}$ and $C_{14:0}$ was not significantly affected by CMC. CMC significantly increased the digestibility of $C_{18:2}$ in the broiler chickens given the coconut oil containing diet.

Discussion

The apparent digestibility of total lipid was lower for the birds given the diet containing tallow than for those fed the diets with either soyabean oil or coconut oil. This observation is in agreement with previous findings (Ketels & de Grootte, 1989; Renner & Hill, 1960 and 1961a,b; Wiseman & Lessire, 1987). Tallow is relatively rich in $C_{16:0}$ and $C_{18:0}$ and the absorption of these fatty acids is limited by their rate of incorporation into micelles (Friedman & Nyland, 1980; Shiau, 1981). In the young broiler chicken, a low bile acid concentration in the digesta, leading to a lowered formation of mixed micelles, may limit lipid absorption (Edwards, 1962; Garret and Young, 1975; Gomez & Polin, 1974 and 1976; Krogdahl, 1985). This characteristic of young broiler chickens would explain the lower digestibility of tallow.

The apparent digestibility for the individual fatty acids differed for the three fats. The fatty acids $C_{16:0}$ and $C_{18:0}$ from soyabean oil were absorbed more efficiently than from coconut oil and tallow. In addition, the apparent digestibilities of the $C_{16:0}$ and $C_{18:0}$ fatty acids from tallow were lower as those from coconut oil. The $C_{18:1}$ fatty acids from coconut oil were not as well absorbed as those from soyabean oil or tallow. The absorption of individual fatty acids is determined by the physicochemical properties of the fat. The $C_{18:2}$ in soyabean oil may enhance the absorption of $C_{16:0}$ and $C_{18:0}$ through the formation of larger micelles with more capacity to take up saturated fatty acids in the core (Freeman, 1976). The $C_{16:0}$ and $C_{18:0}$ in tallow are predominantly esterified in the 1 position of the triacylglycerol molecule which lowers their digestibility (Small, 1991). The endogenous contribution of $C_{18:1}$ may have affected the apparent digestibility of this fatty acid most in birds fed the coconut oil diet because their intake of $C_{18:1}$ was low.

As would be expected (Smits et al., 1996), the CMC diet raised the viscosity of the small intestinal digesta and also its liquid fraction. When the ration contained tallow, the digestibility of total lipid was reduced by CMC. The digestibility of

Table 4 Mean apparent digestibility (%) of total lipid and selected fatty acids for broiler chickens fed the experimental diets.

	Tallow		Soyabean oil		Coconut oil			
CMC	-	+	-	+	-	+	SED[1]	Source of variation[2]
Lipid digestibility	76.1[a,3]	66.0[b]	92.8[c]	90.9[cd]	90.4[cd]	89.2[d]	1.6	CMC, Fat type, CMC x Fat type
Apparent fatty acid digestibility								
$C_{12:0}$	-	-	-	-	98.4[a]	97.9[a]	0.2	
$C_{14:0}$	-	-	-	-	93.6[a]	90.0[a]	1.1	
$C_{16:0}$	62.7[a]	52.3[b]	90.6[c]	88.6[c]	75.4[d]	66.0[ad]	5.1	CMC, Fat type
$C_{18:0}$	24.2[a]	8.7[a]	85.2[b]	64.2[c]	71.8[bc]	56.3[c]	9.7	CMC, Fat type, CMC x Fat type
$C_{18:1}$	94.7[a]	90.7[b]	94.8[a]	91.9[b]	69.3[c]	71.2[c]	2.4	Fat type
$C_{18:2}$	94.1[a]	94.0[a]	98.1[b]	97.8[b]	87.8[c]	92.2[a]	1.2	Fat type, CMC x Fat type

[1] SED = Standard Error of Difference.

[2] $P<0.05$.

[3] Means in the same row with different superscript significantly differ ($P<0.05$).

total fat was not significantly affected by CMC addition when either coconut oil or soybean oil was the major source of fat in the diet. However, CMC did reduce the digestibility of $C_{16:0}$ and $C_{18:0}$ irrespective of their source, but the reduction only reached statistical significance for $C_{16:0}$ from tallow and $C_{18:0}$ from soyabean oil. The feeding of CMC did not influence the absorption of $C_{12:0}$ and $C_{14:0}$. These observations provide some general support of our hypothesis that CMC interferes with micelle formation. The digestion of tallow, that is rich in $C_{16:0}$ and $C_{18:0}$, was expected to be more dependent on micelle formation than is soyabean oil, containing high amounts of $C_{18:1}$ and $C_{18:2}$. The digestion of coconut oil may be independent of micelle formation. Our findings are in line with results from other studies using diets rich in soluble and gelling plant NSPs. Ward & Marquardt (1983) demonstrated that replacing fats containing long chain saturated fatty acids ($C_{18:0}$) with polyunsaturated long chain fatty acids ($C_{18:2}$ and $C_{18:3}$) or medium chain saturated fatty acids ($C_{12:0}$) resulted in a much greater increase in weight gain and feed conversion efficiency of broiler chickens fed rye-based diets than in those fed wheat-based diets. Rye contains more soluble and gelling NSPs than does wheat (Choct & Annison, 1990). Choct & Annison (1992b) noted that in broiler birds fed diets with different levels of isolated arabinoxylans, the ileal and faecal digestibility of saturated long-chain fatty acids was significantly depressed whereas there was no effect on the unsaturated long-chain fatty acids.

It is concluded that the effect of fibre viscosity on lipid digestibility is dependent on the type of dietary fat. Our findings imply that eliminating the viscous properties of NSPs in broiler diets should improve the nutritive value of fats rich in long-chain saturated fatty acids.

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Chapter 5

The antinutritive effect of a carboxymethylcellulose with high viscosity in broiler chickens is not associated with mucosal damage

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Abstract

The condition of jejunal and ileal mucosa was examined to study whether the inhibitory effect of fibre viscosity on lipid digestibility in growing chickens is associated with damage of the small intestinal mucosa. Two semi-purified diets were prepared and fed to growing chickens from 7 to 18 days of age. To one diet a non-fermentable carboxymethylcellulose (CMC) with high viscosity was added. Lipid digestibility was depressed in the birds fed the diet containing CMC ($P=0.021$). The villi were significantly higher in the jejunum of CMC fed chickens ($P=0.033$) and the number of goblet cells per 100 μm of villus length was raised ($P=0.082$). There was no significant effect on the proliferation rate of enterocytes in the jejunum and ileum. Neither did the CMC diet significantly affect the composition of mucins in the villi. In contrast to our working hypothesis, the results indicate that CMC may have had a beneficial instead of a detrimental effect on the condition of the small intestinal mucosa. The effect of gelling fibres on the condition of the mucosa may be dependent also on their fermentability. Luminal events must be held responsible for the lowered lipid digestibility in broiler chickens fed the diet containing highly viscous CMC.

Introduction

There is sufficient evidence that soluble arabinoxylans and ß-glucans present in barley, wheat and rye depress fat digestibility, when they are included in high levels in broiler chicken diets (Antoniou *et al.*, 1981; Choct & Annison, 1990; Fengler & Marquardt, 1988; White *et al.*, 1981) and that the antinutritive effect of these soluble NSPs is associated with an increase in digesta viscosity (Annison, 1993; Bedford & Classen, 1992; Choct & Annison, 1992). Smits *et al.* (1996a) noted that non-fermentable dietary carboxymethylcellulose (CMC) with high viscosity raised digesta viscosity and depressed lipid digestibility in broiler chickens compared to CMC with low viscosity. It was concluded that fibre viscosity *per se* is involved in the antinutritive effect on lipid digestibility in broiler chickens.

Recently, Viveros *et al.* (1994) observed morphological changes in the jejunum of broiler chickens fed barley-based diets compared with birds fed corn-soy diets. They noted a shortening and thickening of the villi and an increment in the number of goblet cells in birds fed the barley diet. The barley diet reduced lipid digestibility. It was suggested that changes in mucosal morphology might be involved in modification of small intestinal nutrient absorption by ß-glucans present in barley. The addition of a ß-glucanase enzyme preparation counteracted the mucosal modifications and produced an improvement in digestibility of lipid and starch and animal performance. Similar results have been seen in chicks fed rye based diets with a high content in gelling arabinoxylans, where the rate of epithelial turnover was significantly increased compared to maize fed controls and addition of a xylanase preparation reduced turnover to almost equivalent levels as the control (Smithard & Silva, cited by Bedford, 1996).

It is established that nutrient absorption may be affected by mucosal atrophy. The presence of shorter and more tongue-shaped instead of finger-shaped villi may reduce the effective surface for in particular lipid absorption that essentially takes place at the tip of the villi (Bézard & Bugaut, 1986; Caspary, 1992). Moreover, increased crypt cell proliferation may result in less mature cells covering the villi. The absorptive functions of these are less developed, thus lowering the ability to absorb lipids (Hampson, 1986; Parsons, 1986). Finally, increased crypt cell proliferation may alter the number of goblet cells and may change their mucin composition (Koninkx *et al.*, 1988). This may lead to changed physicochemical properties of the mucus layer, which is an important

barrier for lipid absorption (Smithson *et al.*, 1981; Wilson & Dietschy, 1974).

Because soluble and gelling plant NSPs present in barley and rye are readily fermentable (Carré, 1991), it is difficult to asses separately the effects of viscosity and fermentability. Dietary non-fermentable CMC with high viscosity was therefore used to study whether the antinutritive effect of fibre viscosity on lipid digestibility in broiler chickens is associated with damage of the small intestinal mucosa.

Materials and methods

Animals and diets

In total 144 female Ross bred broiler chickens were obtained from Cobroed, Lievelde, The Netherlands. All birds were given at arrival a spray with freeze dried bacteria in water (0.5% w/v) derived from adult hens (Broilact, Orion Corporation, Turku, Finland). The birds were housed in 12 cages with wire-mesh floors. Each cage housed 12 broiler chickens. The room temperature was controlled at 32°C directly after hatching (at day zero) and gradually reduced to 22°C at the end of the trial. The birds were given a 24-hour light schedule. Chickens had free access to food and water.

Two experimental diets were prepared, a control diet and a diet containing 1% of CMC (Table 1). From day 0 to day 7 all the chickens received the control diet. At day 7, six cages were randomly allocated to the control diet and the other six cages to the CMC diet. The excreta were collected from day 15 to 18 and stored at -20°C. The excreta were pooled per cage, freeze dried and milled using a 1 mm sieve.

Collection of samples

At day 18, one chicken per cage was intracardially euthanized with T61® (2 ml, with 0.2 g butramide, 0.05 g mebezonicumiodide and 0.005 g tetracaine-hydrochloride per ml; Hoechst, Veterinär GmbH, München, Germany) and the

Table 1 The composition of the experimental diets (%)

	Control	CMC
Corn	34.0	34.0
Corn starch	30.0	30.0
Soybean protein isolate	20.0	20.0
Animal fat	5.0	5.0
Ground limestone	1.0	1.0
Monocalcium phosphate	1.4	1.4
Potassium bicarbonate	1.0	1.0
DL-methionine	0.2	0.2
Vitamin-Mineral premix[1)]	1.25	1.25
CMC[2)]	0	1.0
Cellulose[3)]	6.0	5.0
Chromium oxide	0.15	0.15

[1)] The vitamin/mineral premix supplied per kg diet (mg): thiamin, 1; riboflavin, 6; calcium pantothenate, 12; niacin amide, 40; pyridoxine, 2; cyanocobalamin, 0.0225; choline chloride, 369; folic acid, 1; biotin, 0.065; retinyl acetate, 25; cholecalciferol, 0.05; Dl-α-tocopheryl acetate, 32.5; menadione, 1.8; ascorbic acid, 20; MgO, 995; MnO_2, 134; $ZnSO_4H_2O$, 155; $FeSO_4H_2O$, 233; $CuSO_45H_2O$, 50; $Na_2SeO_35H_2O$; 0.6; $CoSO_47H_2O$, 0.6; KI, 4; ethoxyquin, 100.

[2)] Carboxymethylcellulose of high viscosity (AF 2805, Akzo Chemicals, Arnhem, The Netherlands)

[3)] Cellulose type BWW 40 (Rettenmaier und Söhne, Ellwangen/Jagst, Germany)

small intestine was taken out. For the histological measurements one jejunal and ileal segment of 10 cm in length were taken from the middle of the jejunum and the ileum. The jejunum was defined as the part from the end of the duodenum to the Meckels diverticulum, and the ileum as the part from the Meckels diverticulum to the caeca. The two segments were rinsed with 10 ml of ice-cold 0.9% NaCl, cut longitudinally at the mesenteric attachment and Swiss rolls were made. After fixation of the tissue in 0.01 M phosphate-buffered 4% formalin, pH = 7.2, for periods in excess of 48 h, dehydration and embedding in paraffin, sections of 5 μm were cut.

Analyses and measurements

Chromium in 1 g samples of feed and freeze dried ileal chyme and excreta were determined by atomic absorption spectrophotometry (SpectrAA-10, Varian Nederland BV, Houten, The Netherlands) after ashing at 550°C and subsequent

oxidation with potassium bromate and a solution of magnesium sulphate and orthophosphoric acid. Total lipid contents of feed and excreta were determined by extraction of the samples with hexane after boiling in hydrochloric acid (3M) for 30 min. The apparent lipid digestibility was calculated using the following formula: $DC_{Lipid} = (1 - [(Cr_{diet}/Cr_{excreta})x(Lipid_{excreta}/Lipid_{diet})]) \times 100$, where DC_{Lipid} = apparent digestibility coefficient (%), $Cr_{diet,excreta}$ = concentration of chromium in diet or excreta and $Lipid_{diet,excreta}$ = concentration of lipids in diet or excreta.

Histological characterization of the jejunal and ileal mucosa was performed in sections cut at right angels to the surface of the mucosa. Fifteen well oriented villi and crypts per jejunal and ileal sample were then measured at 100x magnification by means of the TEA Image Manager System (Difa Measuring Systems B.V., Breda, The Netherlands). The height of the villus was represented by the distance from the crypt opening to the tip of the villus. The crypt depth was determined from the base of the crypt to the level of the crypt opening. In addition, the villus/crypt ratio was calculated. Ten crypts were used to determine the number of mitoses (meta- and anaphases) per crypt. The number of goblet cells was counted in 15 well oriented villi using Alcian Blue-Periodic Acid Schiff (AB-PAS) stained sections (Mowry, 1956). The average values were used to calculate the mean value of each parameter in the control and experimental groups.

In order to characterize the mucin composition of the villus goblet cells, the sections were either stained with AB-PAS to discriminate between acid and neutral mucins or with High Iron Diamine-Alcian Blue (HID-AB; Spicer, 1965) to separate the acid mucins into sulphomucins and sialomucins. The percentage of differently stained goblet cells was determined in 10 well-oriented villi in each sample of the jejunum and the ileum.

Statistical analysis

The level of statistical significance was pre-set at $P<0.05$. ANOVA was used to identify statistically significant differences between the control group and the CMC treatment.

Results

Lipid digestibility was significantly depressed in birds fed the CMC diet

($P=0.021$, Table 2). The villi were longer in the jejunum of CMC-fed chickens ($P=0.033$). No significant effect was found on crypth depth or the number of mitoses per crypt. In birds fed the CMC diet, the villi contained significantly more goblet cells ($P=0.006$) and the number of goblet cells per 100 μm villus length

Table 2 Lipid digestibility and villus length, crypt depth, number of goblet cells and number of mitoses in the jejunum and ileum of broiler chickens fed either the control or CMC diet.

	Control	CMC	SED	Level of significance[1]
Lipid digestibility	76.1	67.8	2.4	0.021*
Jejunum				
Villus length, μm	841	984	48	0.033*
Crypt depth, μm	156	168	18	>0.500
Villus/crypt ratio	5.49	5.92	0.56	0.471
Number of villus goblet cells	96	123	7	0.006**
Number of crypt goblet cells	40	47	4	0.149
Number of goblet cells per 100 μm of villus length	11.4	12.9	0.7	0.082
Number of goblet cells per 100 μm of crypt depth	26.4	28.0	2.1	0.481
Number of mitoses per 100 μm of crypt length	7.8	6.4	0.9	0.207
Ileum				
Villus length, μm	575	632	32	0.148
Crypt depth, μm	142	131	17	>0.500
Villus/crypt ratio	4.16	4.93	0.73	0.338
Number of villus goblet cells	121	122	7	>0.500
Number of crypt goblet cells	52	52	1	>0.500
Number of goblet cells per 100 μm of villus length	21.2	19.4	1.6	0.321
Number of goblet cells per 100 μm of crypt depth	37.1	40.6	4.1	0.431
Number of mitoses per 100 μm of crypt length	7.8	6.6	0.6	0.098

[1]Level of significance: *$P<0.05$; **$P<0.01$.

was nearly significantly raised ($P=0.082$). The CMC diet had no significant effect on villus length, crypt depth, number of mitoses in the crypt or the number of goblet cells and goblet cell density in the ileum (Table 2).

No significant differences were found between the control and CMC-fed

chickens in the percentage of goblet cells containing acid, neutral or a mixture of acid and neutral mucins. Neither was there a significant difference in the percentage of goblet cells containing either sulphomucins or sialomucins. The CMC fed birds had significantly less goblet cells containing sulpho- plus sialomucins in the ileum ($P=0.005$, Table 3).

Table 3 Histochemical composition of mucins in goblet cells of jejunal and ileal villi of broiler chickens fed either the control or CMC diet

	Control	CMC	SED	Level of significance[1]
Jejunal goblet cells				
Acid mucins, %	19.9	15.2	3.4	0.229
Neutral mucins, %	2.6	1.7	0.5	0.177
Acid plus neutral mucins, %	77.5	83.1	3.4	0.168
Sialomucins, %	30.3	18.0	7.7	0.176
Sulphomucins, %	53.8	67.0	10.2	0.267
Sialo- plus sulphomucins, %	15.9	15.1	2.9	>0.500
Ileal goblet cells				
Acid mucins, %	12.3	11.6	1.3	>0.500
Neutral mucins, %	2.2	2.4	0.8	>0.500
Acid plus neutral mucins, %	85.5	86.0	1.3	>0.500
Sialomucins, %	33.2	27.6	8.5	>0.500
Sulphomucins, %	47.3	57.7	9.4	0.330
Sialo- plus sulphomucins, %	19.5	14.7	1.0	0.005**

[1]Level of significance: **$P<0.01$.

Discussion

In accordance with previous findings, the CMC diet depressed lipid digestibility in the broiler chickens (Smits et *al.*, 1996a). However, in contrast to the findings of Viveros et *al.* (1994) and Smithard & Silva (cited by Bedford, 1996) using

respectively barley and rye based diets with high levels of gelling, fermentable NSPs, the diet with non-fermentable CMC did not affect the small intestinal mucosa in broiler chickens of similar age. On the contrary, the observed longer villi and the tendency towards a lower mitotic activity in the jejunum indicate an improved condition of the mucosa in the CMC fed broiler chickens. Similar findings were observed in the ileum, although less pronounced. The suggested beneficial effect is also supported by the higher density of goblet cells observed in the jejunal villi. The presence of more goblet cells may indicate a decreased secretive activity of mucins as a result of a diminished turnover of the mucus layer on the mucosal surface, which fits into the findings of longer villi and a lowered mitotic rate. Moreover, these data are in accordance with the tendency towards a lower proportion of sialomucin containing goblet cells and higher proportion of sulphomucin containing goblet cells. Sulphate esters appear to be associated with long and branched carbohydrate chains, whereas sialic acids are associated with short and more linear oligosaccharide side chains (Mantle & Allen, 1989), which may indicate the presence of more mature goblet cells with a lower turnover of their mucins.

It is plausible that CMC may have contributed to the protective properties of the mucus layer. CMC is highly viscous (lubricating) and in contrast to mucins not degradable by digestive enzymes and microbes. Soluble and gelling plant NSPs like arabinoxylans in rye or ß-glucans in barley are well fermentable (Carré, 1991). These NSPs, present at the surface of or within the mucus layer, may serve as microbial substrate and may stimulate bacterial proliferation and attachment of bacteria to the mucins and glycocalix. Subsequently, bacteria may cause atrophy of the villi as is observed by Viveros *et al.* (1994) in broiler chickens fed barley-based diets. The suggestion that the effect of gelling fibres on mucosal condition may be dependent on their fermentability is supported by the observations of Gee *et al.* (1996). Guar gum, that is viscous and fermentable, raised crypt cell proliferation rate in the small intestine of rats, whereas non-viscous, fermentable lactitol or viscous, non-fermentable hydroxypropyl methylcellulose had no effect.

There are also indications that the intestinal bacteria play an important role in mediating the effect of gelling NSPs on lipid digestibility in broiler chickens (Annison, 1993, Smits & Annison, 1996). Smits *et al.* (1996b) suggested that CMC may lower lipid digestibility in broiler chickens by reducing the concentration of bile salts in the digesta and by bacterial transformation of bile salts. They observed a significant raise in microbial numbers in the small intestine. The

lower concentration of bile salts and deconjugation of bile salts will reduce their efficacy to solubilise lipids (Hofmann & Mysels, 1992). These luminal events may have caused the observed antinutritive effect of CMC on lipid digestibility.

It can be concluded that the antinutritive effect of gelling fibres on lipid digestibility is not primarily associated with mucosal damage. In contrast, non-fermentable CMC with high viscosity depressed lipid digestibility but tended to improve the condition of the mucosa.

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Chapter 6

The inhibitory effect of carboxymethylcellulose with high viscosity on lipid absorption in broiler chickens coincides with reduced bile salt concentration and raised microbial numbers in the small intestine.

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Abstract

Two diets, with or without a non-fermentable carboxymethylcellulose (CMC) with high viscosity, were fed to broiler chickens of 2 wks old to study whether the anti-nutritive effect of gelling fibres on lipid digestibility may be associated with reduced intestinal bile salt concentration. Moreover, the microflora was examined to study whether possible changes in bile salt concentration coincide with alterations in microbial numbers. CMC depressed apparent lipid digestibility ($P=0.021$). Feed intake and weight gain were not significantly affected. Water intake was raised in CMC fed birds ($P=0.039$). Bile acid concentration in small intestinal digesta was lowered ($P=0.047$) in birds receiving the CMC diet, which may have been caused by the raise in water content of digesta ($P<0.001$). The amount of bile acids per g dry matter or per mg chromium was not reduced in small intestinal contents. Broiler chickens fed the CMC diet lost more bile acids with the excreta ($P<0.001$). Total aerobic and anaerobic microbial counts in the intestinal digesta were significantly raised in the duodenum plus jejunum ($P=0.038$) but not in the ileum. Significant increments were found in the numbers of *Clostridiae* ($P=0.017$), *Lactobacilli* ($P=0.009$), *Bacteroides* ($P=0.022$) and yeasts and moulds ($P=0.012$). The present study supports the hypothesis that a non-fermentable gelling fibre (CMC) lowers apparent lipid digestibility by reducing the concentration of bile acids in the chyme in broiler chickens. Moreover, the ingestion of gelling fibres may raise the bacterial activity in the small intestine, which may further contribute to malabsorption of lipids.

Introduction

Soluble and gelling fibres present in wheat, barley and rye like arabinoxylans and β-glucans reduce nutrient digestibility in broiler chickens (Choct & Annison, 1990; Fengler & Marquardt, 1988; White *et al.*, 1981). Smits *et al.* (1996a) recently demonstrated, by using non-fermentable carboxymethylcellulose of varying viscosity, that a raise in digesta viscosity *per se* may lead to a reduced apparent digestibility of protein, starch and in particular lipids. They proposed that an increase in digesta viscosity may impair the hydrolysis and/or the solubilisation of lipids. In addition, they suggested that modification of the activity of the intestinal flora may participate in mediating the antinutritive effect of fibre viscosity on lipid digestibility.

The inhibitory effect of fibre viscosity on lipid absorption has been shown to be dependent on the type of fat. The absorption of fats composed of saturated long chain fatty acids is more affected by gelling fibres than of those composed of polyunsaturated or medium chain fatty acids (Smits *et al.*, 1996b; Ward & Marquardt, 1983). These observations are in accordance to a reduced efficacy of bile salts to solubilise lipids, since particularly the saturated long chain fatty acids depend on solubilisation by bile salts for their efficient absorption (Finley & Davidson, 1980). In young broiler chickens, the low bile acid concentration in the digesta is considered as the most limiting factor for lipid absorption (Krogdahl, 1985). If the concentration is lower than the so called critical micellar concentration, mixed micelles formed by bile salts spontaneously dissociate (Freeman, 1969). Moreover, the small intestinal microflora may metabolise bile salts (Eyssen & van Eldere, 1984). This will reduce their efficacy to solubilise lipids (Hofmann & Mysels, 1992). Thus, modifications of the small intestinal microflora may contribute to the antinutritive effect.

Normally, bile salts are efficiently absorbed in the distal small intestinal tract and return to the liver. Only a small amount of bile acids escapes absorption and is excreted via the faeces. However, bacterial transformations of bile salts in the small intestine may reduce their absorption and the amount that is returned to the liver. Consequently, more bile acids are excreted (Eyssen & van Eldere, 1984). This interruption of the enterohepatic recycling of bile acids may lower the concentration of bile acids in the bile because young broiler chickens have a limited capacity to synthesise bile salts, therefore, to adapt to increased losses (Serafin & Nesheim, 1970). This may lower the concentration of bile acids in the

chyme. Alternatively, bile salt excretion may be raised by gelling fibres due to binding or sequestering of bile salts (Vahouny *et al.*, 1980; Story & Kritchevsky, 1976).

A study was therefore undertaken to examine whether the anti-nutritive effect of fibre viscosity on lipid digestibility is associated with a lower concentration of bile acids in the digesta and/or increased faecal bile acid output. Furthermore, we investigated the modifications of the microflora in the small intestine since this may affect the recycling of bile salts and their efficacy to solubilise lipids.

Materials and methods

Animals and diets

One day old, female broiler chickens (Ross, Cobroed, Lievelde, The Netherlands) were housed in 12 cages with wire-mesh floors. Each cage housed 12 chickens. All birds were given at arrival a spray with freeze dried bacteria in water (0.5% w/v) derived from adult hens to standardize the flora (Broilact, Orion Corporation, Turku, Finland). The birds were exposed to constant light and given free access to pelleted feed and water. Room temperature was controlled at 32°C directly after hatching (at day zero) and gradually reduced to 22°C at the end of the trial.

Two experimental diets were prepared, a control diet and a diet containing 1% of carboxymethylcellulose (CMC, Table 1). At day 7 six cages were randomly allocated to the control diet and the other six cages to the CMC diet (Table 1). The experimental diets were fed from day 7 to day 18. Body weights were determined at the beginning and at the end of the experiment. Feed and water intake were recorded.

Collection of samples

Excreta were collected daily from day 15 to 18 and stored at -20°C. The excreta were pooled per cage, freeze dried and milled using a 1 mm sieve for the determination of bile salts, lipids and chromium. At day 18, 5 birds per cage were randomly selected for the collection of the small intestinal digesta. The birds were killed by an intracardial injection with 2 ml T61® containing 0.2 g butramide, 0.05 g mebezonicumiodide and 0.005 g tetracainehydrochloride per ml (Hoechst Veterinär GmbH, München, Germany). Immediately after injection

the digestive tract was taken between the gizzard and the ileocecal junction. Intestinal contents were collected by gently finger stripping the intestinal segments. During collection, the intestinal contents were stored on ice. The contents were pooled per segment per cage, stored at -20°C and subsequently freeze dried and milled using a 1 mm sieve for the determination of chromium and bile salts.

Also at day 18, one bird per cage was randomly selected for the determination of the microflora in the small intestinal tract. Immediately after euthanisation, the digestive tract between the gizzard and Meckel's diverticulum was removed and considered as duodenum plus jejunum. The ileum was isolated as the section between Meckel's diverticulum and the ileocecal junction.

Table 1 The composition of the experimental diets (%)

	Control	CMC
Corn	34.0	34.0
Corn starch	30.0	30.0
Soybean protein isolate	20.0	20.0
Animal fat	5.0	5.0
Ground limestone	1.0	1.0
Monocalcium phosphate	1.4	1.4
Potassium bicarbonate	1.0	1.0
DL-methionine	0.2	0.2
Vitamin-Mineral premix[1)]	1.25	1.25
CMC[2)]	0	1.0
Cellulose[3)]	6.0	5.0
Chromium oxide	0.15	0.15

[1)] The vitamin/mineral premix supplied per kg diet (mg): thiamin, 1; riboflavin, 6; calcium pantothenate, 12; niacin amide, 40; pyridoxine, 2; cyanocobalamin, 0.0225; choline chloride, 369; folic acid, 1; biotin, 0.065; retinyl acetate, 25; cholecalciferol, 0.05; Dl-α-tocopheryl acetate, 32.5; menadione, 1.8; ascorbic acid, 20; MgO, 995; MnO_2, 134; $ZnSO_4H_2O$, 155; $FeSO_4H_2O$, 233; $CuSO_45H_2O$, 50; $Na_2SeO_35H_2O$; 0.6; $CoSO_47H_2O$, 0.6; KI, 4; ethoxyquin, 100.

[2)] CMC of high viscosity (AF 2805, Akzo Chemicals, Arnhem, The Netherlands)

[3)] Cellulose type BWW 40 (Rettenmaier und Söhne, Ellwangen/Jagst, Germany)

Analyses

Chromium in samples of diets and freeze dried ileal chyme and excreta was determined by atomic absorption spectrophotometry (SpectrAA-10, Varian Nederland BV, Houten, The Netherlands) following the method of Costigan & Ellis (1987) after the samples had been digested as described by Williams *et al.*, (1962). Total lipid contents of feed and excreta were determined by extraction of the samples with hexane (50819, Boom, Meppel, The Netherlands) after boiling in hydrochloric acid (3M) for 30 minutes. The apparent lipid digestibility was calculated using the following formula: $DC_{Lipid} = (1 - [(Cr_{diet}/Cr_{excreta})x(Lipid_{excreta}/Lipid_{diet})]) \times 100$, where DC_{Lipid} = apparent digestibility coefficient of lipids, $Cr_{diet,excreta}$ = chromium concentration in diet or excreta and $Lipid_{diet,excreta}$ = lipid concentration in diet or excreta.

Bile salts in excreta were extracted according to Grundy *et al.* (1965) and enzymatically quantified according to Kalek *et al.* (1984). The enzymatic method used, involves dehydrogenation of the 3-α-hydroxy bile salts by 3-α-hydroxysteroid dehydrogenase, and, therefore, does not quantify bile salts which are sulphated at the 3-α-position. However, control experiments in which samples of digesta and faeces were subjected to solvolysis (Princen *et al.*, 1990), prior to enzymatic quantification, indicated that no significant amounts of sulphated bile salts were present.

For total aerobic and anaerobic viable counts, the two small intestinal segments including the contents were suspended in 225 ml of physiological saline (0.9% w/v NaCl in distilled water) immediately after removal and subsequently homogenised with an Ultra Turrax (T25). After homogenising, tenfold dilutions were made and from the appropriate dilutions 20 μl was suspended on agar plates. The following selective media were used: sterile defibrinated sheep blood agar (PCH-Diagnostica, Haarlem, The Netherlands) for total aerobic and anaerobic counts, Levine Eosin Methylene-Blue (LEMB) for *Escherischia coli*, Kanamycine Aesculine Azide (KAA) agar for *Streptococci*, Mannitol Salt Agar (MSA) for *Staphylococci*, Sobouraud dextrose (SAB) for yeasts and moulds, Reinforced Clostridial Agar (RCA) for *Clostridiae*, de Man Rogosa Sharpe (MRS) agar for *Lactobacilli* and Bacteroides Bile Esculine (BBE) for *Bacteroides*. The selective media for the microbial counts of total aerobic, *E. coli*, *Staphylococci*, *Streptococci* and yeasts and moulds were incubated aerobically for 1 day at 37°C. The agar plates for the counts of total anaerobic, *Clostridiae*, *Lactobacilli* and *Bacteroides* were incubated for 2 days in an aseptic environment

at 37°C. The anaerobic incubation was carried out in aseptic jars (Anaero Jar, Oxoid, Unipath Ltd., Basingstoke, England). Anaerobic environment in the jars was created by using AnaeroGen sachets (AN25, Oxiod). The anaerobic environment was controlled by using an oxygen indicator (BR55, Oxoid). The microbial numbers were expressed as $\log_{10}$ colonic forming units (cfu) per g sample.

Statistical analyses

Data were analyzed by ANOVA. Differences between birds fed the control and CMC diet were significant at $P<0.05$. Levels of significance are: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Results

The CMC diet raised the water intake ($P=0.039$, **Table 2**). Weight gain, feed intake and feed to gain ratio were not significantly affected.

The apparent digestibility of lipids was reduced in birds fed the CMC diet ($P=0.021$, **Table 3**). The bile acid concentration in small intestinal digesta was lower in these birds. However, the bile acid content in the dry matter and the bile acid to chromium ratio were not significantly reduced. The bile acid excretion per g dry matter and per mg chromium was higher in broiler chickens receiving the CMC diet ($P=0.001$).

The total aerobic ($P=0.038$) and anaerobic ($P=0.045$) viable counts in the proximal small intestine were significantly raised, as well as the numbers of yeasts and moulds ($P=0.012$), *Clostridiae* ($P=0.017$), *Lactobacilli* ($P=0.009$) and *Bacteroides* ($P=0.022$). There were no significant differences in microbial numbers in the distal small intestine (**Table 4**).

Discussion

The present study was undertaken to determine whether the effect of CMC on lipid digestibility is associated with a reduced concentration of bile salts in the small intestine. Indeed, the CMC diet lowered the concentration of bile acids in the digesta, and consequently, may have significantly decreased the solubilisation

of lipids. Ketels (1991) reported, by relating the apparent digestibility of tallow in broiler chickens of different ages with the concentration of bile acids in their small intestinal digesta, that the bile acid concentration accounted for 68% of the variation in the digestibility of lipids. A concentration of less than 70 μmol bile acids per g fat free matter was associated with a reduction in the digestibility of tallow. The lipase concentration in the digesta had no significant relation with lipid digestibility. Neither was there a significant correlation between the bile acid concentration and the apparent digestibility of soyabean oil. Thus, the lowered bile acid concentration may have limited the absorption of particularly the saturated long chain fatty acids (Smits *et al.*, 1996b).

The significant raise in water content and the increased loss of bile salts with the excreta may both have contributed to the lowered bile salt concentration in the small intestine of CMC fed birds. Because CMC is non-fermentable (Smits *et al.*, 1996a), CMC maintains it water-holding properties during passage through the intestinal tract and may have impaired therefore the resorption of water. Moreover, it has been suggested that CMC raises the osmolality of the digesta by

Table 2 The weight gain, feed intake, water intake and lipid digestibility in broiler birds fed either the control or CMC diet from 7 to 18 days of age.

	Control	CMC	SED	Level of significance[1]
Weight gain, g	435	429	7	0.438
Feed intake, g	593	597	7	>0.500
Water intake, g	1195	1290	33	0.039*
Feed to gain ratio	1.365	1.392	0.016	0.168

[1]Level of significance: * $P<0.05$.

the presence of more non-absorbed nutrients of low-molecular weight which also may impair water absorption (van der Klis, 1993).

The increased loss of bile salts with the excreta indicates that less bile salts were returned to the liver and/or that more bile salts were sequestered or binded

Table 3 Apparent digestibility of lipids and bile salt contents in small intestinal digesta and excreta of broiler chickens fed either the control or CMC diet.

	Control	CMC	SED	Level of significance[1]
Apparent lipid digestibility, %	76.1	67.8	2.4	0.021
Water content digesta, %	76.5	79.7	0.6	<0.01**
Bile salt content digesta				
- μmol/g digesta	14.4	11.7	1.0	0.047*
- μmol/g dm	66.0	62.2	4.3	0.428
- μmol/mg Cr	10.0	11.3	0.8	0.180
Bile salt content excreta				
- μmol/g dm	13.4	15.8	0.2	<0.001***
- μmol/mg Cr	1.62	2.14	0.03	<0.001***

[1]Level of significance: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

by CMC. Possibly, the broiler chickens fed the CMC diet were not capable to adapt to the increased losses by synthesising and secreting more bile salts as the concentration in the digesta was lowered. However, the bile acid to chromium ratio in the digesta, which is an indicator for the amount of bile salts that was present per g of feed, was not lowered. In contrast, the ratio even tended to be higher. The increased bile salt to chromium ratio may indicate that CMC reduced the absorption of bile salts (Ebihara & Schneeman, 1989) and/or increased the biliary secretion. A stimulation of the pancreatic-biliary secretion has been noted in rats fed diets with gelling fibres (Ikegami *et al.*, 1990) and broiler chickens fed a diet containing isolated arabinoxylans (Angkanaporn *et al.*, 1994). Notwithstanding, the broiler chickens fed the CMC were not capable to adapt to such an extent that the concentration of bile salts in the digesta was equal to the birds fed the control diet.

CMC increased the microbial counts in the small intestine. This observation could have been secondary to the impaired digestion and absorption of nutrients (Smits *et al.*, 1996a). In addition, however, it could also have contributed to inactivation and faecal loss of bile salts.

Table 4 Microbial counts (log_{10} cfu) in proximal and distal small intestine in broiler chickens fed either the control or CMC diet.

	Control	CMC	SED	Level of significance[1]
pH digesta				
- proximal	6.6	6.6	0.2	>0.500
- distal	7.9	7.2	0.3	0.050*
Total aerobic				
- proximal	6.4	7.5	0.4	0.038*
- distal	7.6	7.8	0.4	>0.500
Escherischia coli				
- proximal	5.7	7.2	0.7	0.101
- distal	7.7	7.9	0.3	>0.500
Staphylococci				
- proximal	4.9	5.1	0.3	>0.500
- distal	5.8	5.5	0.5	>0.500
Streptococci				
- proximal	5.3	5.5	0.4	0.481
- distal	6.6	6.4	0.5	>0.500
Yeasts & moulds				
- proximal	6.6	7.7	0.3	0.012*
- distal	7.8	8.3	0.4	0.281
Total anaerobic				
- proximal	6.6	7.6	0.4	0.045*
- distal	8.0	8.0	0.3	>0.500
Clostridiae				
- proximal	6.5	7.6	0.3	0.017*
- distal	7.7	8.2	0.4	0.356
Lactobacilli				
- proximal	6.5	7.6	0.3	0.009**
- distal	7.7	8.1	0.4	0.288
Bacteroides				
- proximal	6.5	7.6	0.3	0.022*
- distal	7.7	8.2	0.3	0.170

[1]Level of significance: * $P<0.05$; ** $P<0.01$.

Increased bacterial activity in the proximal small intestine may lead to deconjugation of bile acids. Bile acid deconjugation may be mediated by various

Gram-positive genera such as *Enterococci*, *Lactobacilli*, *Clostridiae* and *Bacteroides* (Hylemon, 1985). These bacteria produce a conjugated bile salt hydrolase which liberates the glycine and/or taurine moiety from the side chain of the steroid core. Upon hydrolysis, the physicochemical properties of bile acids change drastically (Hofmann and Mysels, 1992). The unconjugated bile acids are less effective in forming micelles. When a complex flora becomes established in the proximal small intestine, malabsorption of fat may be the result (Gracey, 1979; King & Toskes, 1979). Thus, the increased bacterial numbers in birds fed the CMC diet may have contributed to the lowered lipid digestibility as well.

The results of the present study suggest that fibre viscosity may lower lipid digestibility in broiler chickens by reducing the effective concentration of bile acids in the digesta. Moreover, the ingestion of gelling fibres may raise the microbial activity in the small intestinal tract, which may further contribute to malabsorption of lipids.

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Chapter 7

Lipid digestibility and plasma cholesterol in conventional and germfree rats fed a carboxymethylcellulose with high viscosity

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Abstract

A diet with 3% of a high viscosity carboxymethylcellulose (CMC) instead of cellulose was fed to conventional and germfree rats to study the role of the microflora in the effect of a gelling fibre on lipid digestibility and plasma cholesterol. Rats with a microflora were obtained by inoculating germfree rats with a specific pathogen free (SPF) flora. Microbial counts in segments of the proximal and distal small intestine of SPF rats were carried out to examine whether CMC modifies the small intestinal flora. CMC had no effect on weight gain and feed intake but raised the water intake ($P<0.05$). The apparent digestibility of $C_{18:1}$ and $C_{18:2}$ was significantly reduced by CMC ($P<0.05$). In addition, the apparent digestibility of total lipids and of total C_{18} fatty acids tended to be lower in rats receiving the CMC diet ($P=0.08$). However, the magnitude of the effect of CMC on lipid digestibility tended to be greater in conventional compared to germfree rats. Plasma cholesterol levels were reduced in rats fed the CMC diet ($P<0.05$), particularly in germfree rats. The microbial numbers of *Lactobacilli* in the proximal small intestine were lower in rats fed the CMC containing diet ($P<0.05$) and tended to be higher in the distal small intestine ($P=0.07$). Also the total aerobic ($P=0.08$) and anaerobic flora ($P=0.05$) tended to be raised in the distal small intestine. In addition, length of the small intestine ($P<0.05$) and weight of the caecum ($P<0.001$) were raised in CMC fed rats.The increase in length of the small intestine, the enlarged caecum and the raise in microbial numbers indicate that more non-absorbed nutrients have reached the distal small intestine in CMC fed rats. The results of the present study suggest that the effect of a CMC with high viscosity on lipid digestibility, but not on plasma cholesterol, is mediated by the presence of the intestinal flora.

Introduction

Certain soluble and gelling fibres such as guar gum, pectin, psyllium and cellulose derivates lower lipid absorption and reduce plasma cholesterol (Anderson, 1990). These effects are associated with their viscosity (Carr *et al.*, 1996; Ebihara *et al.*, 1979; Judd & Truswell, 1985; Smits *et al.*, 1996a). Gelling fibres may lower the rate of hydrolysis and absorption of nutrients, including lipids, which has been demonstrated *in vitro* (Edwards *et al*, 1988; Isaksson *et al.*, 1982) and *in vivo* in rats (Johnson & Gee, 1981; Tinker & Schneeman, 1989), hamsters (Carr *et al.*, 1996) and humans (Jenkins *et al.*, 1978; Wood *et al.*, 1994). The mechanism of this effect may involve a delay in the diffusion of bile salts (Ebihara & Schneeman, 1989). This would particularly lower the digestibility of lipids that depend on the formation of micelles, such as saturated long chain fatty acids. Smits *et al.* (1996) demonstrated in broiler chickens fed a diet with 1% carboxymethylcellulose (CMC) of high viscosity, that a raise in digesta viscosity only reduced the apparent lipid digestibility of tallow, with mainly long chain saturated fatty acids, but not of soyabean oil or coconut oil with high contents in polyunsaturated or medium chain fatty acids (Smits *et al.*, 1996b).

In addition to the delayed diffusion of bile acids, the mechanism may be mediated by alterations of the intestinal microflora. An increase of microbial numbers in the small intestine has been observed when broiler chickens were fed diets with gelling NSPs (Salih *et al.*, 1981; Smits *et al.*, 1996c; Wagner & Thomas, 1978). The possibility that the microflora is involved is supported by the following observations. Supplementation of diets containing pectin or rye with antibiotics substantially raised lipid digestibility (Wagner & Thomas, 1978). Smits and Annison (1996) noted that in germfree broiler chickens the detrimental effect of highly viscous CMC on lipid digestibility was negligible, whereas there was a marked increase in digesta viscosity. However, this study did not include conventional chickens as controls. Finally, Campbell *et al.* (1983) found that the reduction in lipid digestibility in broiler chickens fed rye-based diets could effectively be alleviated by maintaining the broiler chickens in a germfree environment. These results indicate that the microflora may mediate the lipid lowering effects of gelling fibres in broiler chickens. The mode of action is not clear, but it is suggested that a raise in microbial activity in the small intestine may lead to deconjugation of bile acids which subsequently may

lower their efficacy to formate micelles (Hofmann and Myssels, 1992).

In the present study we tested the hypothesis that the effect of CMC with high viscosity on lipid digestibility is mediated by the intestinal flora. We therefore compared the effects of CMC in conventional and germfree rats. In addition, plasma cholesterol levels were examined to study whether the effect of CMC on plasma cholesterol is associated with the effect on lipid digestibility.

Materials and methods

Animals and diets

In total 24 germfree (Wistar/Cpb: WU) rats (Central Animal Laboratory, University of Nijmegen, The Netherlands) of three months old, were used and randomly allocated to four groups of six animals. Two groups maintained germfree and were housed in three plastic isolators with in each isolator two rats of each treatment. The other two groups of six animals were inoculated twice with a specific pathogen free flora (SPF) with an interval of 3 days. All rats were housed individually in polypropylene cages at a temperature of 20 °C with a 12 hour light-dark cycle (lights on from 6 am to 6 pm) . The animals had free access to sterile feed and water. The diets had been irradiated (20 kRay) and vacuum-packed under sterile conditions (Gammaster, Ede, The Netherlands). In the preliminary period of 7 days, all rats received the control diet (**Table 1**). Subsequently, the rats either continued to receive the control diet or were given the diet with 3% CMC. Body weights were determined at the beginning and at the end of the experimental period of 12 days. Feed and water intake were recorded. The germfree status of gnotobiotic rats was verified by means of microscopic examination of Gram-stained faecal smears and microbial culturing techniques of faecal samples at the end of the experiment.

Collection of samples

During the last 4 days of the experimental period the faeces of the each rat was collected and stored at -20 °C. The faeces was pooled per rat, freeze dried and milled using a 1 mm sieve for the analysis of chromium, lipid and bile salt contents. At the end of the experiment all rats were anesthetized by diethyl ether inhalation and then killed by cervical dislocation. Before euthanisation,

Table 1: Composition of the experimental diets (g/kg)

Ingredients	CMC -	CMC +
Corn	315.6	315.6
Cornstarch	315.5	315.5
Casein	150	150
Animal fat	80	80
Cholesterol	5	5
Calcium carbonate	12.4	12.4
Sodium phosphate	15.1	15.1
Potassium chloride	7	7
Magnesium carbonate	1.4	1.4
Vitamin premix[2]	36	36
Mineral premix[2]	10	10
Chromium oxide	2	2
Cellulose[3]	50	20
CMC[4]	0	30

[1]Contents of total lipids and selected fatty acids (g/kg): lipid, 101; $C_{16:0}$, 19.1; $C_{16:1}$, 1.8; $C_{18:0}$, 9.5; $C_{18:1}$, 32.8; $C_{18:2}$, 14.6; $C_{18:3}$, 0.9.

[2]The vitamin and mineral premix supplied per kg diet (mg/kg):
thiamin, 12; riboflavin, 9; calcium pantothenate, 53.4; niacin amide, 60; pyridoxine, 18; biotin, 6; folic acid, 3; choline chloride, 6000; cyanocobalamin, 0.15; *Dl*-α-tocopheryl acetate, 180; menadione, 0.15; retinyl acetate, 24; cholecalciferol, 6; $FeSO_4.7H_2O$, 174; MnO_2, 79: $ZnSO_4.H_2O$, 33; $NiSO_4.6H_2O$, 13; NaF, 2; KI, 0.2; $CuSO_4.5H_2O$, 15.7 ; $Na_2SeO_3.5H_2O$, 0.3; $CrCl_3.6H_2O$, 1.5; $SnCl_2.2H_2O$, 1.9; NH_4VO_3, 0.2: maize starch, 39307.5.

[3]Cellulose type BWW40 (Rettenmaier und Söhne, Ellwangen/Jagst, Germany)

[4]CMC of high viscosity (AF2805, Akzo Chemicals, Arnhem, the Netherlands).

blood samples were taken by heart puncture for the determination of cholesterol, triglyceride and bile acid levels in the plasma. The abdomen was opened, and the entire small intestine and colon were removed. The length and weight of the small intestine and the weight of the caecum with contents were determined. The segment from the stomach to the middle of the small intestine was considered as the proximal small intestine and the segment from the middle to the caecum as the distal small intestine. Segments of 15 cm were taken in the middle of the proximal and distal small intestine for the determination of

microbial numbers.

Analyses

Excreta were pooled per rat, freeze dried, ground (1 mm sieve) and mixed. Chromium was determined in duplicate following the method of Costigan & Ellis (1987) after the samples had been digested as described by Williams *et al.* (1962). Total lipid contents of the diets and excreta were determined gravimetrically by extraction of the samples with hexane using a Sohxlet apparatus, following boiling in hydrochloric acid (3 M) for 30 minutes. Fatty acid analysis was performed essentially according to the procedures NEN 5410a and NEN 5410b (NNI, 1996). Methyl esters of free fatty acids were separated using a gas chromatographer (GC MEGA 8160-OS, Fisons Instruments, Breda, The Netherlands) equipped with on-column injection and flame ionisation detection (FID) over a fused silica capillary column (DB-23, J+W Scientific, Folsom, USA) by using temperature programming. Helium was used as carrier gas. Heptadecanoic acid ($C_{17:0}$) was added to each sample as an internal standard. The apparent digestibilities of total lipid and selected fatty acids were calculated as: $DC_{diet} = (1-[(Cr_{diet}/Cr_{excr}) \times (L,F_{excr}/L,F_{diet})]) \times 100$ where DC_{diet} = apparent digestibility of either total lipid (L) or fatty acids (F) in the diet; Cr_{diet} = concentration of chromium in the diet; Cr_{excr} = concentration of chromium in the excreta; L,F_{excr} = concentration of either total lipid or fatty acids in the excreta; L,F_{diet} = concentration of either total lipid or fatty acids in the diet.

Bile salts in faeces were extracted according Grundy *et al.* (1965) and then enzymatically quantified according to Kalek *et al.* (1984). The enzymatic method used, involves dehydrogenation of the 3α-hydroxy bile salts by 3α-hydroxysteroid dehydrogenase, and, therefore, does not quantify bile salts which are sulphated at the 3α-position. However, control experiments in which samples of digesta and faeces were subjected to solvolysis (Princen et al, 1990), prior to enzymatic quantification, indicated that no significant amounts of sulphated bile salts were present. Serum bile acids were determined by enzymatic quantification (Enzabile, Nycomed AS, Oslo, Norway). Triglyceride and cholesterol in the plasma were determined with the use of commercial test combinations (CHOD-PAP and GPO kits, Boehringer-Mannheim GmbH, Mannheim, Germany).

For total aerobic and anaerobic microbial counts, the two small intestinal

segments including their contents were suspended in 225 ml of physiological saline (0.9% w/v NaCl in distilled water) immediately after removal and subsequently homogenised with an Ultra Turrax (T25). The samples for the anaerobic viable counts were prepared in an aseptic environment. After homogenising, tenfold dilutions were made of which 20 μl was suspended on agar plates. Sterile defibrinated blood agar was used for total aerobic and anaerobic counts, Levine EMB (LEMB) agar for *Enterobacteriaceae*, Kanamycine Aesculine Azide agar (KAA) for *Streptococci*, Mannitol Salt Agar (MSA) for *Staphylococci*, Sabouraud dextrose (SAB) agar for yeasts and moulds, Reinforced Clostridial Agar (RCA) for *Clostridiae*, de Man Rogosa Sharpe (MRS) agar for *Lactobacilli* and *Bacteroides* Bile Esculine (BBE) for *Bacteroides*. The agar plates for the microbial counts of total aerobic, *Enterobacteriaceae*, *Streptococci*, *Staphylococci*, yeasts and moulds were incubated aerobically for 1 day and the agar plates of *Clostridiae*, *Lactobacilli* and *Bacteroides* were incubated anaerobically for 2 days at 37 °C. The anaerobic incubation was carried out in an aseptic environment using jars. The anaerobic environment was controlled by using an oxygen indicator (BR55, Oxoid). The microbial counts were expressed as $\log_{10}$ colonic forming units (cfu).

Statistical analyses

The data were subjected to analysis of variance. The generalised model in the analysis of variance was $Y_{ij} = \mu + M_i + D_j + (MxD)_{ij} + e_{ijk}$, where Y_{ij} = observation, μ = mean, M_i = Microbial status, D_j = Diet and e_{ijk} = residual variation. Levels of significance are given: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Results

The CMC diet raised the water intake in SPF and germfree rats ($P=0.036$) but had no effect on weight gain or feed intake (**Table 2**).

The weight of the caecum relative to body weight was significantly raised in germfree rats ($P<0.001$) compared to their conventional counterparts (**Table 3**). The diet with CMC raised caecum weight ($P<0.001$) and increased the relative length of the small intestine ($P<0.05$).

The apparent digestibility of total lipids, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:2}$ and

Table 2 Final body weight, total weight gain, feed intake and water intake[1] of conventional and germfree rats fed a diet with or without CMC.

Microbial status	CMC	Final body weight g	Weight gain g	Feed intake g	Water intake g
Conventional	-	355.8	43.7	399.8	416.5
Conventional	+	345.5	35.0	317.2	494.0
Germfree	-	362.2	32.2	310.5	451.2
Germfree	+	363.0	31.3	304.3	485.2
SED		4.7	2.4	5.5	12.3
Level of significance[2]:					
Microbial status		0.223	0.142	>0.500	>0.500
CMC		>0.500	0.351	>0.500	0.036*
Microbial status x CMC		>0.500	0.433	0.308	0.389

[1] Experimental period of 12 days.
[2] *$P<0.05$

Table 3 Weights of the intestinal segments with contents, pancreas and liver and length of small intestine in conventional and germfree rats fed a diet with or without CMC.

Status	CMC	Small intestine rel. weight % of b.w.	Small intestine rel. length cm/100 g b.w.	Caecum rel. weight % of b.w.	Liver rel. weight % of b.w.
Conventional	-	2.408	30.7	1.105	4.651
Conventional	+	2.516	31.7	1.568	4.535
Germfree	-	2.390	29.5	2.194	4.691
Germfree	+	2.767	32.3	3.120	4.444
SED		0.063	0.4	0.060	0.069
Level of significance[1]:					
Microbial status		0.373	>0.500	0.001***	>0.500
CMC		0.073	0.026*	0.001***	0.203
Microbial status x CMC		0.311	0.257	0.074	>0.500

[1] *$P<0.05$; ***$P<0.001$

total C_{16} fatty acids were significantly lower in SPF rats compared to germfree rats ($P<0.001$). The apparent digestibility of $C_{18:1}$ and $C_{18:2}$ was significantly reduced by CMC ($P<0.05$). In addition, the apparent digestibility of total lipids and of total C_{18} fatty acids tended to be lower in rats receiving the CMC diet ($P=0.08$, **Table 4**).

Germfree rats had lower bile acid ($P<0.001$) and higher cholesterol ($P<0.001$) levels in blood plasma (**Table 5**). Also the cholesterol content in the liver was significantly higher ($P<0.001$). The diet with CMC lowered plasma triglyceride ($P=0.043$) and plasma cholesterol levels ($P=0.046$). This effect was more pronounced in germfree rats. Moreover, the CMC diet lowered the cholesterol content of the liver ($P=0.029$). The bile acid excretion per mg of chromium was lower in CMC fed conventional rats, whereas the CMC diet

Table 4 Apparent digestibility of total lipids and selected fatty acids in conventional and germfree rats fed a diet with or without CMC.

Microbial status	CMC	Total lipids %	$C_{16:0}$ %	$C_{16:1}$ %	$C_{18:0}$ %	$C_{18:1}$ %	$C_{18:2}$ %	Total C_{16} %	Total C_{18} %
Conventional	-	87.2	91.2	100.3	80.5	99.0	99.9	92.0	96.1
Conventional	+	85.3	88.4	99.9	74.7	98.4	99.4	89.4	94.7
Germfree	-	89.9	93.8	99.3	86.4	98.5	98.9	94.2	96.6
Germfree	+	89.1	93.6	98.2	87.4	97.7	98.4	94.1	96.2
SED		0.3	0.5	0.1	1.0	0.1	0.1	0.4	0.3
Level of significance[1]:									
Microbial status		0.001***	0.001***	0.001***	0.001***	0.071	0.001***	0.001***	0.077
CMC		0.080	0.134	0.139	0.264	0.032*	0.044*	0.127	0.083
Microbial status x CMC		0.425	0.164	>0.500	0.119	>0.500	>0.500	0.169	0.336

[1] *$P<0.05$; ***$P<0.001$

raised it in germfree rats (interaction microbial status x CMC: $P<0.05$). Conventional rats fed the CMC diet tended to have lower serum bile acid levels, whereas germfree rats fed this diet tended to have higher levels (interaction microbial status x CMC, $P=0.078$).

Conventional rats fed the CMC diet had lower microbial counts of *Lactobacilli* in the proximal intestine ($P<0.03$) whereas higher values were obtained in the distal small intestine ($P=0.07$). Moreover, the number of total aerobic and anaerobic counts tended to be higher in the distal small intestine (respectively $P=0.08$ and $P=0.05$, **Table 6**).

Discussion

The results show that the presence of the microflora reduces the apparent digestibility of lipids. Moreover, the CMC diet tended to reduce the apparent lipid digestibility in conventional rats more than in germfree rats which supports

Table 5 Bile salt excretion, serum bile salts and triglyceride and cholesterol levels in plasma and liver in conventional and germfree rats fed a diet with or without CMC.

Microbial status	CMC	Bile salts excreta/ mg Cr	Bile salts serum µmol/l	Triglycerides plasma mmol/l	Cholesterol plasma mmol/l	Cholesterol liver mmol/g
Conventional	-	1.83	33.7	2.75	2.64	40.1
Conventional	+	1.69	24.6	2.41	2.58	32.7
Germfree	-	2.50	8.4	2.84	3.72	73.5
Germfree	+	2.75	12.6	1.96	3.14	68.9
SED		0.04	1.8	0.14	0.08	1.3
Level of significance[1]:						
Microbial status		0.001***	0.001***	>0.500	0.001***	0.001***
CMC		0.473	0.496	0.043*	0.046*	0.029*
Microbial status x CMC		0.018*	0.078	0.358	0.107	0.500

[1] *$P<0.05$; ***$P<0.001$

the hypothesis that the effect of CMC on lipid digestibility is, at least partially, influenced by the microflora. The more detrimental effect of CMC on lipid digestibility in conventional rats than in germfree rats is also reflected by the reduced digestibility of in particular the long chain fatty acids $C_{16:0}$ and $C_{18:0}$. However, it cannot be excluded that bacterial hydrogenation of unabsorbed, unsaturated fatty acids may have raised the amount of $C_{16:0}$ and $C_{18:0}$ and lowered the concentration of $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ in the digesta which influences the apparent digestibilities of these fatty acids (Eyssen & Parmentier, 1974). Support for this possibility can be derived from the results in the germfree rat, which had a higher digestibility of $C_{16:0}$ and $C_{18:0}$ but slightly lower digestibility of $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$. Notwithstanding, the lower apparent absorption of the total group of C_{16} and C_{18} fatty acids indicate a reduced absorption of lipids in CMC fed conventional rats.

The effects of CMC in the present study, however, were of a lesser magnitude than those observed in broiler chickens, where lower concentrations (1%) of the same type of CMC reduced the apparent digestibility of lipids by 10 to 30% (Smits *et al.*, 1996a, 1996b and 1996c). This observation indicates that the process of lipid digestion in young adult rats is less sensitive to modification by gelling fibres. Broiler chickens have relatively low concentrations of bile acids in the small intestinal contents (Green and Kellog, 1987) and there is evidence that this limits lipid absorption (Krogdahl, 1985). Supplementation of broiler chicken diets with bile acids significantly improved fat digestion (Edwards, 1962; Gomez and Polin, 1974). Differences between bile salt pool sizes and/or composition between the rat and the broiler chicken may be responsible for the quantitative different effects of CMC in both species.

CMC significantly reduced plasma cholesterol in germfree rats, and to a lesser extent in conventional rats. Several mechanisms are proposed to explain the hypocholesterolemic effects of gelling fibres (Anderson, 1990). The results of this experiment allows to exclude some of the proposed mechanisms. The hypocholesterolemic effect of gelling fibres could not have been the result of inhibition of cholesterol synthesis by absorbed short chain fatty acids because in germfree rats fermentation is absent. Theoretically, dietary cholesterol absorption could be reduced by CMC (Carr et *al.*, 1996). However, the absorption of long chain saturated fatty acids was not affected by CMC and, according to Vahouny *et al.* (1983), the correlation between absorption of these fatty acids and cholesterol in rats is high (0.96). The raise in serum bile acid

Table 6 Microbial numbers (log_{10} cfu) in the proximal and distal small intestine of conventional rats fed a diet with or without CMC.

	CMC			
	-	+	SED	Level of significance[1]
Total aerobic				
proximal	7.93	7.47	0.14	0.140
distal	8.53	8.93	0.10	0.082
Total anaerobic				
proximal	8.10	7.73	0.13	0.184
distal	8.70	9.17	0.10	0.052
Enterobacteriaceae				
proximal	4.13	3.97	0.35	>0.500
distal	3.90	4.82	0.29	0.156
Staphylococci				
proximal	3.17	3.13	0.21	>0.500
distal	3.78	3.50	0.23	>0.500
Streptococci				
proximal	2.97	2.83	0.42	>0.500
distal	2.60	2.73	0.45	>0.500
Lactobacilli				
proximal	8.23	7.57	0.13	0.027*
distal	8.63	9.02	0.09	0.073
Clostridiae				
proximal	7.83	7.52	0.12	0.235
distal	8.58	8.77	0.19	>0.500
Bacteroides				
proximal	7.87	7.35	0.14	0.092
distal	8.50	8.77	0.16	0.419

[1] $*P<0.05$

in germfree rats may have affected the synthesis of cholesterol in the liver (Heuman et *al.*, 1988). Moreover, CMC may have lowered plasma insulin levels like other gelling fibres do (Jenkins et *al.*, 1978), but the reported effects of ambient insulin levels on plasma cholesterol are controversial (Anderson, 1990; Lewis & Steiner, 1996). Thus, it is not clear how CMC did lower plasma cholesterol in germfree rats and whether a single mechanism is responsible in both, conventional and germfree rats.

Since CMC is non-fermentable (Smits *et al.*, 1996a) it maintains it water-holding properties during passage through the intestinal tract. This may impair water resorption and may raise the amount of water that enters the caecum (Van der Klis *et al.*, 1993). In addition to this, a delay in nutrient absorption may also result in a more bulk and undigested material reaching the distal small intestine and entering the caecum (Blackburn & Johnson, 1981; Wyatt *et al.*, 1986). We suggest that this effect has led to an increased length of the small intestine and weight of the caecum in germfree rats in the present study. This is supported by the observations of Pell *et al.* (1995), who observed a trophic effect on small intestinal length and increased caecal weight in germfree mice fed a diet with guar gum. The CMC diet tended to lower the microbial counts in the proximal small intestine whereas the effect was opposite in the distal part. This may also have been the result of substrate availability. Viscous polysaccharides delayed the disappearance of starch from the small intestine and increased the amount reaching the distal small intestine and the caecum in rats (Tinker & Schneeman, 1989).

The results of the present study suggest that the microflora may mediate the effect of gelling fibres on lipid absorption. The significant reduction in plasma cholesterol in germfree rats and the absence of an effect on apparent digestibility of total lipids indicate that the plasma cholesterol lowering effect of CMC is independent of lipid absorption and is not mediated by the intestinal microflora.

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Chapter 8

A plea for a specified prediction of the nutritive value of carbohydrates in poultry diets

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Abstract

The outline of a working model is presented for the qualification of carbohydrates in poultry rations according to their utilisation and their effects in the gastrointestinal tract. For assessing the nutritional value, the carbohydrates that will be digested by host derived enzymes must be distinguished from those that are fermented by the microflora. Energy losses during the fermentation process and a lower efficiency of utilisation of the produced volatile fatty acids compared to glucose reduce the energetic value of fermented carbohydrates. For assessing the so called antinutritive properties of α-galactosides and non-starch polysaccharides (NSPs), these carbohydrates are characterised physicochemically by their solubility and viscosity in the gastrointestinal tract. The soluble, non-viscous α-galactosides and insoluble, non-viscous NSPs appear to have a minor and/or inconsistent impact on feed intake and growth rates of broiler chickens. However, the soluble NSPs with gelling properties such as soluble arabino-xylans, ß-glucans and pectins have distinctive antinutritive properties due to various changes in the small intestine. These include an increase in viscosity of the liquid phase of the digesta, a delayed hydrolysis and absorption of end products of the digestion process, and increased bacterial activity. Moreover, the amount of endogenous material in the digesta may be raised. For the evaluation of the antinutritional effects it is therefore necessary to quantify the gelling properties of feed ingredients. Next, dose-response relationships should be described to derive a threshold level for diet formulation. The qualification of carbohydrates presented in this paper may be a helpful tool for a more specified prediction of the nutritive value of carbohydrates in poultry diets.

Introduction

In raw materials for poultry diets, the carbohydrate fraction is commonly characterised by the crude fibre content and by the nitrogen free extract residue (NFE). The NFE is calculated as the dry matter weight of the sample minus the analyzed contents of crude ash, crude protein, crude fat and crude fibre in the Weende analyses. In most western countries, the apparent metabolizable energy (ME) content of the carbohydrate fraction in raw materials for poultry diets is then calculated by the following formula: AME carbohydrates (kJ) = 17.32 x digestible NFE (g/kg). Crude fibre is assumed to be indigestible (Subcommittee Energy WPSA, 1988). However, from a chemical and physiological point of view this characterisation of the carbohydrate fraction may not be adequate for the assessment of its ME value (Smits and Annison, 1996). First, the NFE and crude fibre determinations are not chemically defined and may contain some non-carbohydrate components. The energy content depends on the carbohydrate composition. Secondly, it does not discriminate between carbohydrates that are digested by host derived enzymes and those that are fermented by the microflora. Thirdly, the interaction with the apparent digestibility of proteins and lipids are not well predicted by the NFE or crude fibre levels. Some carbohydrates do have 'antinutritive' properties in poultry that may affect the ME-value of the feed. Various soluble non-starch polysaccharides (NSPs) with gelling properties impair the digestibility of lipids, protein and starch in the broiler chicken (White *et al.*, 1981; Fengler and Marquardt, 1988; Choct and Annison, 1990; Choct and Annison, 1992a).

The present study discusses the nutritional value of the various carbohydrates in raw materials for poultry diets from their chemical and physical properties.

The carbohydrate composition

Carbohydrates can be classified chemically as mono-, di-, oligo- and polysaccharides. The latter can be subclassified into starch and non-starch polysaccharides (NSPs). These different classes of carbohydrates each have different chemical structures (**Table 1**).

Table 1: Chemical classification of carbohydrates and main representatives in poultry diets.

Carbohydrate	Chemical structure
Disaccharides	
sucrose	α-1,2 linked glucose and fructose
Oligosaccharides	
(DP[1] = 3 - 9)	
α-galactosides	α-1,6 linked galactose to the glucose moiety of sucrose
Non Starch Polysaccharides	
(DP ≥ 10)	
arabinoxylans	ß-1,4-linked xylopyranosyl residues with terminal 1,2 and 1,3 arabinofuranosyl substitions
ß-glucans	ß-1,4-linked glucosepolymer with ß-1,3 side linkages
cellulose	ß-1,4-linked glucosepolymer
pectin	α-1,4-linked, partially methylated, galacturonic acid residues
other NSPs	complex NSP structures, with amongst others, mannose and galactose residues
Starch	amylose (α-1,4-linked glucosepolymer) and amylopectin (α-1,4-linked glucosepolymer with α-1,6-side linkages)

[1]DP: degree of polymerisation

The main sugars present as monosaccharides in poultry diets are glucose and fructose. The dietary contents of these monosaccharides as free sugars however is in general very low and normally less than 1% (Carré, 1993). These monosaccharides and other monosaccharides, like xylose or arabinose, can also be released from NSPs by the inclusion of NSP degrading enzymes. Sucrose is the major disaccharide with common dietary levels of 2 -3% (Carré, 1993). High levels are found in particular in molasses. Significant levels of sucrose are also found in soybeanmeal, rapeseedmeal and sunflowerseedmeal. The major oligosaccharides in poultry diets are the α-galactosides raffinose, stachyose and verbascose. The dietary contents of α-galactosides range usually from 0.5 to 1.5%. In particular soybeanmeal, lupins, peas, rapeseedmeal and sunflowermeal do contain high levels of α-galactosides (Saini, 1989). The non-starch polysaccharides (NSPs) can represent also a considerable group of carbohy-

drates. In poultry diets the levels may vary from 5 up to 15%. Arabinoxylans, ß-glucans and cellulose essentially form the NSP fraction in rye, barley, wheat and maize, including byproducts of these cereals (Bach-Knudsen, 1993; Smits and Annison, 1996). Barley and oats contain high NSP-levels compared to maize and wheat. In peas, soybeanmeal, sunflowerseedmeal and rapeseedmeal the NSP composition is rather complex and does contain pectic substances (Bach-Knudsen, 1993; Smits and Annison, 1996). Starch is the major carbohydrate source in poultry diets with dietary levels ranging from 30 to 45%. The chemical composition of the carbohydrate fraction in important feed ingredients is given in **Table 2.**

Table 2 Approximate carbohydrate composition of some important ingredients in poultry diets (% of dm).

	Maize	Wheat	Barley	Rye	Oats	Soy-bean meal	Sunfl.-seed-meal	Rape-seed-meal	Peas	Tapioca
Sucrose[1,2]	1	1	1	2	1	7	3	6	2	<1
α-Galactosides[1,2]	<1	<1	<1	<1	<1	6	2	3	5	<1
Insoluble NSP[2,3,4,5]	6	7	12	10	20	20	25	21	15	4
Soluble NSP[2,3,4,5]	1	1	3	5	4	4	4	6	5	5
Starch[2,4]	70	66	59	61	47	2	1	2	46	75
Total specified	78	77	77	77	72	39	37	41	73	85

Values obtained from Carré (1993)[1]; Bach-Knudsen (1993)[2]; Smits and Annison (1996)[3]; Huygebaert and De Grootte (1995)[4]; Irish & Balnave (1993)[5].

The nutritive value of carbohydrates

For determining the energetic value of carbohydrates the gross energy content, the digestibility, the energy losses during fermentation and the efficiency of utilization of the end products of the digestion process must be considered. Starch and sucrose are broken down by host derived enzymes to the monosaccharides glucose and fructose. Negligible energy losses will occur during the process of digestion and absorption of starch and sucrose. On the other hand, α-galactosides, NSPs and starch indigestible by enzymes of the

alimentary tract may be fermented by the microflora. Energy losses occur by heat loss and gas production and by less efficient utilisation of VFA for either ATP formation or fat deposition compared to glucose. According to Livesey (1992) the proportion of energy from fermented carbohydrates that becomes available to the host (*f*) equals $f = (1\text{-}a\text{-}b\text{-}c) \times g$, where *a* is the proportion of carbohydrate energy lost as microbial mass in the faeces, *b* is the proportion of heat loss with fermentation, *c* is the proportion of energy lost as combustible gas (hydrogen and methane) and *g* is the efficiency of short chain fatty acid metabolism compared to glucose. The energy loss due to fermentation of carbohydrates and less efficient utilisation of volatile fatty acids can be estimated at 30% of the digestible energy content compared to glucose (Black, 1995; Livesey, 1992; Müller and Kirchgessner, 1986). Thus, there is sufficient reason to distinguish the fraction that is digested by host derived enzymes and the fraction that is fermented by the microflora. A similar system has been adopted in pig nutrition (Noblet *et al.*, 1994). The corrected energy values of the main carbohydrate components distinguished in this way are given in **Table 3**.

Table 3 The energy yield of the main carbohydrates.

	Energy content (MJ/kg)[1]	Energy loss fermentation[2]	Corrected energy value (MJ/kg)
Sucrose	16.5	0	16.5
α-Galactosides	16.5	30%	11.6
NSPs and fermented starch	17.5	30%	12.3
Starch	17.5	0	17.5

[1]Heat by combustion, CRC Handbook of Chemistry and Physics (1989)

[2] Black, 1995; Müller & Kirchgessner (1986); Livesey (1992).

Monosaccharides and sucrose

The contribution of free monosaccharides to the energy value of poultry diets is negligible due to the low dietary content. It is also unlikely that sufficient monosaccharides are released by the addition of exogenous enzymes to increase greatly the ME content of the feed or feed ingredient (Chesson, 1992). Moreover, it appears that xylose and arabinose released from arabinoxylans and galacturonic acid released from pectic substances are poorly absorbed and/or

utilised in poultry (Longstaff *et al.*, 1988; Schutte, 1990).

Sucrose is highly digestible in poultry. Carré *et al.* (1995) reported digestibility values of 85% in broiler chickens and 99% in adult cockerels.

α-Galactosides

Like other monogastric species, including humans and pigs, birds do lack the α-galactosidase activity and therefore α-galactosides such as stachyose, raffinose and verbascose are digested through bacterial degradation (Carré,1993). The reported digestibilities of these α-galactosides in poultry vary from 50 to 99% (Brenes *et al.*, 1992; Carré and Lacassagne, 1992; Carré *et al.*, 1995). The addition of exogenous α-galactosidases may improve the nutritional value of the α-galactosides because they cleave galactose of sucrose. The sucrose will be readily utilized. However, the reported effects of α-galactosidases are inconsistent (Brenes *et al.*, 1992; Irish *et al.*, 1995).

Starch

An accurate estimation of the digestibility of the starch component in poultry diets is most important for predicting the energy value of carbohydrates. Although the bird has the appropriate enzymes to breakdown starch to glucose, the starch digestibility may not be complete. Surrounding cell walls, the granule size and the structural arrangement of amylose and amylopectin may prohibit the enzymes to breakdown starch (Moran, 1982). Three main groups have been described for resistant starch (Englyst *et al.*, 1992): physically inaccessible starch (RS_1), uncooked, native starch granules (RS_2) and retrograded starch (RS_3). The latter refers to aggregates that are formed by heat treatment and subsequent cooling of starches. Several authors reported variable starch digestibilities for Australian grown wheats, with values ranging from 80 to 99% (Annison, 1990; Annison, 1991; Mollah *et al.*, 1983; Rogel *et al.*, 1987). Yuste *et al.* (1994) noted that the digestibility of native starches prepared from maize and wheat was about 96% in broiler chickens at the terminal ileum. Pea and cassave starch had a digestibility of resp. 94 and 95%, but bean and potato starch were only digested for 72 and 44%. There was no marked increase in digestibility by fermentation of starch in the caeca in broiler chickens, although the caeca were enlarged in those fed the poorly digestible bean and potato starch. Adult cockerels however had higher digestibilities of bean and potato starch of respectively 94 and 70%. The digestibility of starch can be improved by technological treatments and/or by the addition of NSP-degrading enzymes

(Almirall *et al.*, 1995; Campbell and Bedford, 1992; Classen *et al.*, 1985; Edney *et al.*, 1989; Hesselman and Aman, 1986; Longstaff and McNab, 1987). Supplementation of diets with NSP degrading enzymes reduced the amount of physically inaccessible starch by cell wall disruption and increased the ME value of the carbohydrate fraction (Chesson, 1993; Annison, 1993; Choct *et al.*, 1995). The efficacy of NSP-degrading enzymes on the ME value of the target feedstuff can therefore be introduced in least-cost diet formulation by increasing the amount of digestible starch and consequently, the ME content.

Non-starch polysaccharides

The contribution of the NSP fraction to the ME value of a broiler chicken diet is relatively low. Jørgensen *et al.* (1996) noted that the degradation of NSP constituents was far lower in broiler chickens than found in other animal species such as pigs and rats. It appears that broiler chickens are able to ferment only a part of the soluble fraction of NSPs (Carré, 1992). Poultry with more developed caeca, like layers, cockerels and ducks, are able to ferment more NSPs (Carré *et al.*, 1989). The distinction between the energetic value of starch and fermentable carbohydrates is more important for these birds.

Antinutritive evaluation of carbohydrates

The chemical composition of carbohydrates does not relate unequivocally to its nutritional value. Carbohydrates have physical properties that influences their physiological effects (Eastwood & Morris, 1992). It is recognised that given carbohydrates may have antinutritive properties and depress performance. Roberfroid (1993) and Smits and Annison (1996) reviewed the literature and concluded that carbohydrates should be classified on the basis of their solubility, viscosity and fermentability. This classification may improve the prediction of the physiological effects in monogastric animals and humans. **Table 4** summarizes the reported effects of the physicochemical properties of carbohydrates on various processes in the small intestine of poultry.
The compilation is based on studies with various purified carbohydrates that were classified according to their physicochemical properties.

α-Galactosides

It appears that α-galactosides may depress nutrient digestibility at moderate

Table 4 Physicochemical properties of carbohydrates in relation to their effects in the small intestine of broiler chickens.

	Soluble, non-viscous α-Galactosides	Soluble, viscous NSPs	Insoluble, non-viscous NSPs
	. Stachyose[Sa, Ve] . Raffinose[Sa, Ve]	. Arabinoxylans [Ch, Fe, War] . β-Glucans[Al, Wh, Wan] . Pectin[Dr, Pa, Jo] . Carboxymethylcellulose[Kl, Sm] . Guar gum[Ed, Is]	. Cellulose[Ak, An]
Viscosity digesta	0	⇑[Al, Ch, Dr, Fe, Kl, Sm, Wan, War, Wh]	0
Osmolality digesta	⇑[Wi]	⇑[Kl]	0
Mean retention time digesta	⇓[Wi]	⇑[Kl]	⇓[Ak]
Hydrolysis rate nutrients	0?	⇓[Ed, Fe, Is, Lec]	0?[Ro]
Absorption rate nutrients	0?	⇓[Fe, Jo, Wo]	0?[Ro]
Endogenous secretions	⇑?	⇑[An, Pa, La]	0[An] ⇑[Ak]
Activity intestinal flora	⇑[Sa, Ve]	⇑[Ch, Dr, Pa, Sm]	0⇓?
Dry matter content digesta	⇓[Mo, Ve]	⇓[Kl, Sm]	0?
pH digesta	⇓[Ve]	⇓[Kl] 0[Sm]	0?

References: [Ak]Akiba & Matsumoto (1980) [Al]Almirall *et al.*, 1995; [An]Angkanaporn *et al.*, 1994; [Ch]Choct & Annison, 1992b; [Dr]Drochner *et al.*, 1993 (review); [Ed]Edwards *et al.*, 1988 (in vitro); [Fe]Fengler & Marquardt, 1988; [Is]Isaksson *et al.*, 1982 (in vitro); [Jo]Johnson & Gee, 1981, (rats); [Kl]van der Klis *et al.*, 1993; [Lec]Leclere *et al.*, 1993 (pigs); [Mo]Mollee, 1992 ; [Pa]Parsons *et al.*, 1984; [Ro]Roberfroid, 1993 (review, rats); [Sa]Saini, 1989 (review); [Sm]Smits *et al.*, 1996a; [Ve]Veldman *et al.* (1993); [War]Ward and Marquardt, 1987; [Wan]Wang *et al.*, 1992; [Wi]Wiggins, 1984 (humans, review); [Wh]White *et al.*, 1981; [Wo]Wood *et al.*, 1994.

levels that are commonly found in practical diets (≤1.5%), although the reported effects are controversial. The digestibility of dry matter decreased with more than 1.3% of stachyose and 0.4% raffinose in the diet in adult Leghorn roosters (Leske *et al.*, 1993). A diet with 1.4% raffinose reduced the Protein Efficiency Ratio (PER) significantly in broiler chickens (Leske *et al.*, 1995). Mollee (1992) noted that the apparent ileal digestibility of protein was reduced in cannulated roosters fed a diet with 5% raffinose. However, Trevino *et al.* (1990) did not observe a detrimental effect in broiler chickens fed a diet with 5% of a dried oligosaccharide extract from pea meal (7% raffinose, 23% stachyose and 29% verbascose) .

The antinutritive effect of α-galactosides on nutrient digestion and absorption may be dependent on their osmotic properties and fermentability. Wiggins (1984) noted that the presence of high amounts of non-absorbable low molecular weight sugars may increase the osmolality of the digesta and the amount of water retaining in small intestinal chyme. The raise in volume of fluids may reduce the mean retention time of the digesta in the small intestine and subsequently this may lower nutrient digestibility. Veldman *et al.* (1993) reported that the addition of velasse, the residue after evaporation of an 80% ethanol extract of soybeanmeal generated during the production of soy protein concentrate, had a significant adverse effect on the ileal digestibility of nutrients and resulted in fluid retention and enhanced microbial fermentation in small intestinal contents when fed to piglets.

From a practical point of view it may be important to set a maximum level for the amount of α-galactosides in the diet. Another possibility may be to eliminate the antinutritive properties by the addition of exogenous α-galactosidase enzyme preparations (Brenes *et al.*1993; Irish *et al,* 1995).

Soluble, viscous NSPs

The effect of soluble, viscous NSPs on the performance of poultry is well established. Various authors have reported that the addition of isolated, water-extractable NSPs with gelling properties had a detrimental effect on the growth rate and feed to gain ratio of broiler birds, and also on the digestibilities of lipids, protein and starch (Antoniou *et al.*, 1981; White *et al.*, 1983; Fengler and Marquardt, 1988, Choct and Annison, 1992a). The ingestion of gelling NSPs increased the viscosity of the supernatant of small intestinal contents (Bedford *et al.*, 1991, Bedford and Classen, 1992, Choct and Annison, 1992b). Smits et al. (1996a, b, c and d) used the process of lipid digestion and absorption to highlight the mechanism by which the viscosity of NSPs may affect nutrient digestibility (**Figure 1**). The raise in digesta viscosity, the increase in retention time of digesta and the delay in digestion and absorption of nutrients are proposed as the primary key factors by which fibre viscosity may enhance bacterial activity in the gastrointestinal tract. Subsequently, lipid digestibility may be depressed by a reduced efficacy of bile salts to solubilize lipids. In addition, the hydrolysis of starch and protein and the mucosal uptake of glucose and amino acids is delayed. Moreover, the apparent digestibility of protein may particularly be lowered by an increase in endogenous nitrogen losses (**Table 4**).

It is clear that the intestinal flora is, at least partially, responsible for the

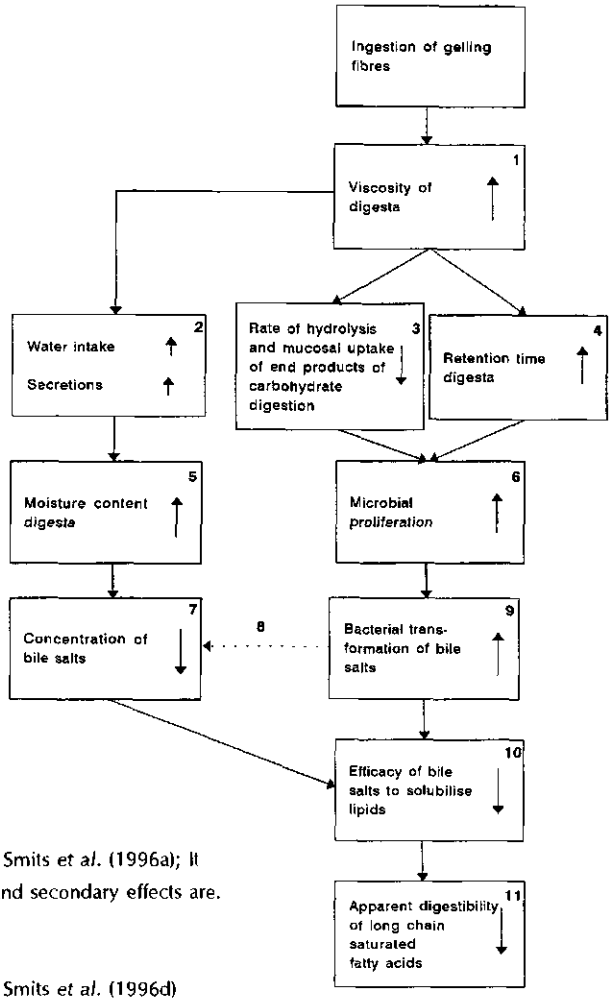
antinutritive effects of gelling NSPs. Smits *et al.* (1996a) demonstrated, by using non-fermentable carboxymethylcellulose with varying viscosity, that the viscosity depressed nutrient digestibility in conventional broiler chickens. However, in germfree broiler chickens, the antinutritive effect of CMC with high viscosity was absent, whereas there was a marked increase in the viscosity of the supernatant of intestinal contents (Smits & Annison, 1996). The role of the microflora in mediating the antinutritive effects of gelling NSPs present in wheat, barley and rye is also noted by other workers (Annison, 1993; Bedford, 1996; Campbell *et al.*, 1983; Choct and Annison, 1992b; Choct *et al.*, 1992; Salih *et al.*, 1991; Wagner and Thomas, 1978).

Gelling and fermentable NSPs present in plant ingredients like arabinoxylans, β-glucans and pectins may be more detrimental than semi-synthetic gelling non-fermentable NSPs like carboxymethylcellulose. Viveros *et al.* (1994) observed in birds fed barley shortening and thickening of the villi, thus affecting the condition of the mucosa. In contrast, a diet with non-fermentable CMC gave an improved condition of the villi (Smits *et al.*, 1996c). It was suggested that gelling plant NSPs present at the surface of or within the mucus layer may serve as microbial substrate and may stimulate bacterial proliferation and attachment of bacteria to the mucus and glycocalix.

The antinutritive effects of gelling NSPs in given feed ingredients or feeds can be monitored by determining the in vitro viscosity. The assay uses the complete ingredient or diet as sample and the viscosity of the supernatant is determined after an *in vitro* digestion procedure. Bedford and Classen (1993) demonstrated that this assay accurately predicted the *in vivo* viscosity of intestinal contents in birds fed a rye based diets with and without enzymes. Such a method may also be valid for predicting the vivo viscosity in chickens fed diets based on wheat (Classen *et al.*, 1995) or barley (Campbell *et al.*, 1989; Rotter *et al.*, 1989).

Given least cost formulations, it is necessary to derive a method capable of predicting the viscosities of mixed diets from the viscosities of the separate ingredients. Recently, Carré *et al.* (1994) described a method for this purpose. They showed that the natural logarithm of the in vitro viscosity values of each ingredient was additive and did provide an accurate prediction of the in vitro viscosity of diets composed of a wide range of ingredients. Formulas were derived to predict the effect of pelleting temperature on in vitro viscosity.
Moreover, enzymes that will lower digesta viscosity allows to raise the content of raw materials with a high level of gelling NSPs (Veldman and Vahl, 1994; Classen and Bedford, 1991). The latter can be taken into account by assessing

Figure 1 The mechanism by which fibre viscosity may depress lipid digestibility in broiler chickens.



[1] See references Table 5.

[2] van der Klis *et al.* (1993); Smits *et al.* (1996a); It is unknown what primary and secondary effects are.

[3] See references Table 5.

[4] See references Table 5.

[5] van der Klis *et al.* (1993); Smits *et al.* (1996d)

[6] Smits *et al.* (1996d,e)

[7] Smits *et al.* (1996d)

[8] Transformed bile salts are less efficiently absorbed. Thus, enterohepatic recycling of bile salts may be lowered, which in turn may lead to reduced secretion of bile salts. The broiler chicken has a limited capacity to synthesise bile salts (Serafin & Nesheim, 1988)

[9] Feighner & Dashkevicz (1988); Coates *et al.* (1981)

[10] Hofmann & Mysels (1992)

[11] Smits *et al.* (1996b)

the effect of enzymes on the in vitro viscosity of the target raw material and subsequently, this value is used in least cost formulation of a diet.

Ideally, the in vitro viscosity value of the diet then accurately predicts the antinutritive effect. However, several diet and animal dependent factors do determine the effect as well. It may therefore be too complex to accurately predict the antinutritive effect. A way to overcome this problem is to assume that there is a threshold level below which the antinutritive effect of viscosity is not evident. The in vitro viscosity of a diet may then not exceed a given threshold value.

Insoluble NSPs

Although insoluble NSPs do have bulking and water holding properties as they can act as a sponge (Cadden, 1987; Parrot & Thrall, 1978; Robertson & Eastwood, 1981) and subsequently may lower the mean retention time of the digesta (Eastwood, 1992; Roberston, 1988), they do not seem to interfere with the process of digestion and absorption like soluble, viscous NSPs (Akiba and Matsumoto, 1980). Only with high inclusion levels performance may be reduced (Jørgensen *et al.*, 1995). It has been suggested that insoluble NSPs with laxative properties might even prove beneficial in situations where there is enhanced bacterial activity in the gut. They may diminish the time available for fermentation in the gut, and bacteria may adhere to the provided insoluble NSP structures (Smits & Annison, 1996; Rogel *et al.*, 1987).

Resistant starch

We do not know whether starch which is not digested by host derived enzymes may have antinutritive effects in poultry. Starches escaping the digestion in the small intestine may enter the caeca and may increase fermentation and enlarge the caeca (Yuste *et al.*, 1991; Coates, 1981). Intestinal bacteria are able to degrade RS_2 and RS_3 (Englyst and Macfarlane, 1986; Wyatt and Horne, 1988; Faulks *et al.*, 1989). However, the fermentation of poorly digestible starches in the hindgut may be limited in poultry (Yuste *et al.*, 1991).

Concluding remarks

The working model described above can be used in practical diet formulation but needs development and validation. Eventually, the specification

of the nutritive value and antinutritive properties of carbohydrates may provide a a more accurate estimation of the value of the carbohydrates in relation to feed utilization and health of poultry.

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SUMMARY

In the nutrition of monogastric farm animals, little attention has been paid to the physicochemical properties of fibres in relation to digestion and absorption of nutrients and gastrointestinal health. Recently, it has been recognised that certain soluble and gelling non-starch polysaccharides (NSPs) depress the digestibility of starch, protein and lipids. This antinutritive effect has been associated with an increase in digesta viscosity. However, the mechanism by which the gelling NSPs affect nutrient digestibility has not been elucidated.

The aim of this thesis was to identify the physicochemical properties of NSPs that are most relevant to the nutrition of the broiler chicken. More specifically, the mechanism by which fibre viscosity may affect nutrient digestibility in broiler chickens has been investigated by using the model compound carboxymethylcellulose (CMC), which is a soluble non-fermentable fibre with high viscosity.

A literature review was conducted to describe the NSPs present in plant ingredients and to identify the most critical physicochemical properties (Chapter 2). It was proposed that the solubility and viscosity of NSPs are the physicochemical properties which are most relevant in terms of an antinutritive effect. The process of lipid digestion was used to highlight the mechanisms by which gelling NSPs may affect lipid digestibility. It was postulated that the ingestion of soluble gelling NSPs raises the viscosity of the digesta and delays the hydrolysis and absorption of lipids. Moreover, it was proposed that modifications of the mucus layer and the mucosa may reduce the absorptive capacity of the small intestine. Finally, it was noted that the microflora may play an important role in mediating the antinutritive effects. In germfree broiler chickens fed diets with varying levels of CMC, a pronounced increase in digesta viscosity did not affect lipid digestibility.

The effect of fibre viscosity *per se* was investigated by using CMC's with varying viscosity (Chapter 3). An *in vitro* fermentation study showed that both, low and high viscous CMC, are non-fermentable. The increase in viscosity of the digesta in birds fed the CMC diet with high viscosity lowered the digestibilities of starch, protein and lipids. Particularly, the apparent digestibility of lipids were depressed. Moreover, birds fed this diet had higher ATP concentrations in their small intestinal chyme, which indicated that the microbial activity had been raised. It was concluded that viscosity *per se* is an antinutritive property of dietary fibre for broiler chickens.

The effect of fibre viscosity on lipid digestibility was further investigated in a study with broiler chickens fed diets containing either tallow, soyabean oil or coconut oil, with or without CMC (Chapter 4). This study was undertaken to test the hypothesis that the solubilisation of lipids may be impaired by the ingestion of gelling fibres. The solubilisation of long chain saturated fatty acids (tallow) is highly dependent on the formation of micelles by bile salts, whereas polyunsaturated fatty acids (soyabean oil) and medium chain fatty acids (coconut oil) do not require bile salts as they are more water-soluble. The results demonstrated that the apparent digestibility of lipids from the tallow-diet was depressed by the CMC diet. However, there was no effect on apparent lipid digestibility in birds fed the soyabean oil or the coconut oil diet. Thus, the effect of fibre viscosity on lipid digestibility is dependent on the type of fat and in particular the digestibility of fats that are composed of saturated long chain fatty acids are depressed.

It has been suggested that gelling plant NSPs may have a detrimental effect on the small intestinal mucosa, which may reduce its capacity to absorb nutrients. The mucosa was examined therefore in broiler chickens fed a diet with CMC (Chapter 5). However, CMC did not damage the mucosa (Chapter 5). In contrast, broiler chickens fed the CMC diet had longer villi in the jejunum and the proliferation rate of crypt cells tended to be lower. Also the composition of the mucins was not significantly changed. The morphological parameters indicated that the condition of the mucosa was improved in CMC fed birds instead of being damaged. It was concluded that luminal factors are primarily responsible for the effect of fibre viscosity on nutrient digestibility and that fibre viscosity does not directly damage the mucosa. It was suggested, however, that in contrast to non-fermentable CMC, gelling plant NSPs may interact with the mucus layer and may serve as substrate for intestinal microbes at the epithelial surface. This may lead to a condition in which the mucosa is damaged.

A lowered concentration of bile salts may be one of the luminal factors that is involved in the impairment of micelle formation in broiler chickens fed diets with gelling fibres (Chapter 6). Birds that received a diet with CMC had a lower bile salt concentration in the digesta, which may have been the result of a significant increase in water content. However, the amount of bile acids per mg chromium in the digesta was not lowered which may indicate that the amount of bile acids per g of feed present in the small intestine was not changed. Because the concentration of bile acids is the most limiting factor for lipid absorption in the young broiler chicken, it is likely that this may explain, at least

partially, the observed lower digestibility of lipids. However, bacterial modification of bile acids may also have contributed to impaired micelle formation. The microbial numbers in the small intestine were significantly raised in CMC fed birds. This may have caused bacterial deconjugation of bile salts with a consequence that these are less effective in forming micelles.

The hypothesis that the effect of fibre viscosity on lipid digestibility is dependent on the intestinal flora was investigated in germfree rats and rats with a specific pathogen free (SPF) microflora (Chapter 7). A diet with 3% CMC reduced lipid digestibility in SPF rats, but the magnitude of the effect was less in germfree rats. Thus, the study indicated that alterations of the microflora may mediate, at least partially, the lipid depressing effect of gelling fibres. However, the magnitude of the antinutritive effect of a diet with CMC on lipid digestibility was less than in broiler chickens. It was suggested that the bile acid concentration in the digesta of rats may not limit lipid absorption. In addition, CMC lowered plasma cholesterol and increased the length of the small intestine in germfree rats. These results indicate that the cholesterol lowering and trophic effects of gelling fibres on the small intestine are not primarily mediated by the microflora.

The results of the review and the studies with CMC were used to describe the outline of a working-concept for a specified prediction of the nutritive value of carbohydrates in poultry diets (Chapter 8). A distinction is proposed between the carbohydrates that are fermented and those that are digested by host derived enzymes. A similar system has been adopted in pig nutrition. The reason is that the energetic value of carbohydrates that are digested by host derived enzymes is estimated to be 30% higher than those fermented by intestinal flora. Moreover, antinutritional effects should be taken into account in the evaluation of the value of carbohydrates. The determination of the *in vitro* viscosity of ingredients may be adequate to predict the *in vitro* viscosity of a diet and subsequently its effect on digesta viscosity. It is proposed that threshold levels should be obtained for the maximum viscosity value of a diet below which antinutritive effects are not evident. The maximum viscosity level in the diet has to be related to the target species. A more specific determination of the nutritive value and antinutritive properties of carbohydrates may provide a more accurate estimation of the value of these carbohydrates in relation to feed utilization and health.

SAMENVATTING

In de veevoeding is tot op heden relatief weinig aandacht besteed aan de invloed van fysisch-chemische eigenschappen van voedingsvezels op de vertering en absorptie van voedingsstoffen en op de gezondheid van de dieren. De aandacht in de veevoeding ging met name uit naar de energetische voedingswaarde. Uit meer recent onderzoek is echter gebleken dat bepaalde oplosbare en visceuze niet-zetmeel polysacchariden (NSPs) uit granen als tarwe, gerst en rogge, de verteerbaarheid van zetmeel, eiwit en vet verminderen bij vleeskuikens. NSPs zijn een belangrijk bestanddeel van voedingsvezels. Het anti-nutritieve effect leek geassocieerd te zijn met een toename van de viscositeit van de darminhoud, hoewel het mechanisme nog onduidelijk was.

De doelstelling van dit proefschrift was het identificeren van de belangrijkste fysisch-chemische eigenschappen van voedingsvezels in relatie tot de vertering en absorptie van nutriënten bij vleeskuikens. Daarbij is specifiek aandacht besteed aan het effect van de viscositeit op de vetvertering. Daartoe is carboxymethylcelullose (CMC) als modelstof gebruikt. CMC is een in water oplosbaar voedingsvezel met hoge viscositeit.

Het literatuuronderzoek werd uitgevoerd om de fysisch-chemische eigenschappen van niet-zetmeel polysacchariden (NSPs) in plantaardige grondstoffen te beschrijven, en om aan te geven welke eigenschappen relevant kunnen zijn voor vertering en absorptie van nutriënten bij vleeskuikens (hoofdstuk 2). Daaruit werd geconcludeerd dat de viscositeit mogelijk de belangrijkste eigenschap is van voedingsvezels met betrekking tot de anti-nutritionele effecten. Het proces van de vetvertering is gekozen om een aantal mogelijkheden te beschrijven, via welk mechanisme visceuze NSPs de vertering kunnen verlagen. Gesteld werd dat een toename in de viscositeit van de darminhoud de snelheid van vertering en absorptie van vet kan verminderen. Daarnaast werd aangegeven dat visceuze NSPs mogelijk een negatief effect hebben op de conditie van het darmslijm en de darmwand waardoor de absorptie van eindprodukten van de vetvertering daalt. Tenslotte werd gesteld dat de microflora in de dunne darm waarschijnlijk een belangrijke rol speelt bij het anti-nutritionele effect. In een oriënterende proef met kiemvrije kuikens die voeders verstrekt kregen met verschillende gehaltes aan CMC, had een toename van de viscositeit van de dunne darminhoud geen effect op de vetvertering.

Het effect van specifiek de viscositeit is bestudeerd door gebruik te

maken van twee typen CMC met een lage en hoge viscositeit die verschilden in gemiddelde polymeerlengte (hoofdstuk 3) en deze te verwerken in proefvoeders voor vleeskuikens. *In vitro* waren beide typen CMC goed oplosbaar en niet fermenteerbaar. Dit was van belang om er zeker van te zijn dat alleen de viscositeit tussen de proefvoeders varieerde en dat het effect van de fermenteerbaarheid van de voedingsvezels werd uitgesloten. Het hoog visceuze CMC verhoogde de viscositeit van de dunne darminhoud en verlaagde de verteerbaarheid van zetmeel, eiwit en vet. Daarnaast werden indicaties gevonden dat de microbiële activiteit in de dunne darm toenam. Geconcludeerd werd dat de viscositeit op zichzelf beschouwd een anti-nutritieve eigenschap is van voedingsvezels voor vleeskuikens.

Het effect van de viscositeit op de vetvertering bij kuikens werd verder bestudeerd met voeders die dierlijk vet, sojaolie of kokosvet bevatten (hoofdstuk 4). De resultaten van de proef gaven aan dat door CMC alleen de verteerbaarheid van het dierlijk vet en van de verzadigde langketen vetzuren werd verlaagd. Daarentegen was er geen effect van de viscositeit op de verteerbaarheid van sojaolie en kokosvet. Geconcludeerd werd dat visceuze NSPs waarschijnlijk de micelvorming verstoren bij vleeskuikens. De slecht wateroplosbare verzadigde langketen vetzuren (voornamelijk aanwezig in het gebruikte dierlijke vet) zijn voor een goede absorptie sterk afhankelijk van micelvorming door galzouten die het vet als het ware oplossen. Onverzadigde langketen vetzuren (met name in sojaolie) en middellangketen vetzuren (met name in kokosvet) zijn beter in water oplosbaar en dus minder afhankelijk van de micelvorming.

Het effect van CMC op de conditie van de darmwand (mucosa) van kuikens werd bestudeerd om na te gaan of veranderingen in de conditie van de darmwand mogelijk bijdragen aan het effect op de vetvertering (hoofdstuk 5). Er werden echter geen aanwijzingen gevonden dat CMC de conditie van de mucosa aantast. Integendeel, de morfologische parameters gaven aanwijzingen dat de conditie was verbeterd in plaats van aangetast. Geconcludeerd werd dat CMC de verteerbaarheid van vet niet verlaagd door aantasting van de mucosa, maar dat veranderingen in de darminhoud verantwoordelijk zijn.

Een verstoorde micelvorming in het vetverteringsproces zou ten eerste het gevolg kunnen zijn van een verlaagde concentratie van galzouten (hoofstuk 6). Het verstrekken van een voer met CMC gaf een verhoogd vochtgehalte in de dunne darm en een verlaagde concentratie aan galzouten, mogelijk als gevolg

van verdunning. Daarnaast werd in de dunne darm waargenomen dat het aantal microben significant toenam. Het is mogelijk dat visceuze NSPs de microbiële activiteit verhogen en dat vervolgens bacteriële deconjugatie van galzouten de effectiviteit van deze galzouten bij de micelvorming verlaagd. Dit zou kunnen verklaren waarom in het oriënterende onderzoek met kiemvrije kuikens geen anti-nutritioneel effect van CMC op de vetvertering werd gevonden. Gesteld werd dat veranderingen in de dunne darmflora mogelijk een belangrijk effect hebben op de vetvertering.

De invloed van de microflora bij het effect van CMC op de vetvertering werd vervolgens bestudeerd in een proef met kiemvrije ratten en met ratten met een SPF flora (hoofdstuk 7). Het effect van CMC op de vetvertering bij SPF ratten was relatief gering t.o.v. het effect bij kuikens. Wel was er een tendens dat het anti-nutritionele effect van CMC op de vetvertering groter was bij conventionele ratten dan bij kiemvrije rattten. De uitkomsten van de studie bevestigen dat de microflora een rol speelt; de effecten waren echter minder uitgesproken dan verwacht. Een verklaring hiervoor is dat ratten voor vet een hogere verteringscapaciteit dan kuikens. Het voer met CMC verlaagde het cholesterolgehalte in het bloedplasma en verlengde de dunne darm bij kiemvrije ratten. Deze resultaten geven aan dat zowel het cholesterolverlagende effect van visceuze voedingsvezels als het effect op de lengte van de dunne darm niet primair door de microflora worden gemedieerd.

De uitkomsten van de studies werden gebruik om een concept te beschrijven dat bruikbaar is voor een meer gespecificeerde voorspelling van de nutritionele waarde van koolhydraten in de voeding van pluimvee (hoofdstuk 8). Ten eerste is het van belang om koolhydraten die verteerd kunnen worden door dier-eigen enzymen, te onderscheiden van de fractie die wordt gefermenteerd. Een vergelijkbaar systeem wordt al toegepast in de varkensvoeding. Daarnaast is het van belang om rekening te houden met mogelijk anti-nutritionele eigenschappen van de niet enzymatisch verteerbare koolhydraten. Met name de α-galactosiden en de NSPs kunnen een probleem vormen. De anti-nutritionele effecten van deze koolhydraten zijn gerelateerd aan hun fysisch-chemische eigenschappen en hun interactie met de microflora in het maagdarmkanaal. De belangrijkste fysisch-chemische eigenschap van de NSPs met anti-nutritieve effecten is de viscositeit. Een systematiek, waarmee uit de viscositeit van ingrediënten de viscositeit van het voer nauwkeurig kan worden voorspeld, is gewenst. Daarbij moeten drempelwaarden worden afgeleid waarboven,

afhankelijk van diersoort, leeftijd en gezondheidsstatus, de viscositeit van het voer niet meer acceptabel is als gevolg van de anti-nutritionele effecten. Een meer gespecificeerd onderscheid in samenstelling en fysisch-chemische eigenschappen van de koolhydratenfractie in grondstoffen zal een betere voorspelling geven van de waarde van deze fractie in relatie tot voederbenutting en gezondheid.

Curriculum vitae

Coen Smits is op 7 april 1964 geboren te Odiliapeel. Na het doorlopen van de lagere school in zijn geboorteplaats behaalde hij in 1982 het VWO-diploma op het Kruisherencollege te Uden. In hetzelfde jaar begon hij aan zijn studie Zoötechniek aan de Landbouwuniversiteit te Wageningen. In 1988 studeerde hij af in de vakken Veevoeding, Bedrijfskunde en Marktkunde. Sindsdien is hij werkzaam als onderzoeker varkensvoeding aan het Instituut voor de Veevoeding 'De Schothorst'. In januari 1991 werd het promotieonderzoek gestart dat als deeltaak werd uitgevoerd. In het kader van dit onderzoek werd een periode van drie maanden doorgebracht bij het Monogastric Research Centre van het Department of Animal Science, Massey University, Nieuw Zeeland.