Effects of Wheat Bran and Pectin Rich Diets on Colonic Metabolism in the Rat.

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ABSTRACT

Two contrasting dietary fibres, wheat bran (10 and 20% w/w) and high-methoxyl pectin (5 and 10%), were added to a purified basal diet as single fibre fractions and as a mixture of 5% wheat bran plus 5% pectin. Pectin is readily fermented whereas wheat bran is relatively resistant to fermentation in the caecum. Unsupplemented basal and test diets were fed for a period of four weeks to adult, male, Albino Wistar rats which had been previously maintained on the stock diet CRM(X). The effect of the diets on colonic metabolism was evaluated from the weight of (wet and dry) caecal contents, caecal-sac, stool weight, short-chain fatty acids (SCFA), water-holding capacity [(WHC) g water/g faecal or caecal material], anaerobic and aerobic viable counts, and bacterial biomass measured as 2-6-diaminopimelic acid (DAPA). 10% pectin increased all the above variables, whereas wheat bran only increased stool weight consistent with the relative resistance to colonic fermentation. Major caecal and faecal SCFA were acetate, propionate and butyrate. 10% pectin increased the molar proportion of caecal acetate and decreased the molar proportion of butyrate. The mixture of wheat bran plus pectin gave a similar caecal SCFA profile to 10% bran. Rats weaned directly onto the experimental diets were compared to adult rats, and the differences in SCFA profiles and concentration observed for the same diet, may be due to adaptation. The manner in which water is held by bacteria and fibre in the colon, determines stool weight. A dialysis method was developed for measuring WHC of colonic contents which gave an insight into the influence of wheat bran and pectin on stool bulk.
To Mum and Dad
DECLARATION

I hereby declare that this thesis does not include work submitted for any degree or professional qualification of this or any other university or institute of learning.

This thesis has been composed by myself and the work described in it is my own (unless otherwise stated) and was carried out in the Wolfson Laboratories, Western General Hospital, Edinburgh (Department of Medicine) between 1984-1987.

ELIZABETH ARMSTRONG
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SUMMARY

1. The colonic metabolism of Canadian Red Spring Wheat bran and high-methoxyl pectin was studied in the male Albino Wistar rat. Adult rats (6 weeks old), which had been maintained on the stock diet (CRM[X]) prior to experimentation, were fed for 4 weeks on a basal diet supplemented with wheat bran (10 and 20% w/w), pectin (5 and 10%) and a mixture of wheat bran (5%) with pectin (5%). In another experiment, rats were weaned directly onto the test diets and fed for 7 weeks. The water-holding capacity (i.e. bound water) of colonic contents was determined using a dialysis method with polyethylene glycol producing an osmotic gradient.

2. Wheat bran produced a significant increase in stool weight of 1g dry weight/g wheat bran fibre intake, and pectin (at 10% level) resulted in a significant increase of 0.1g dry weight/g pectin intake.

3. The bacterial cell wall component, diaminopimelic acid (DAPA), significantly correlated with bacterial numbers \( r=0.45, p<0.05 \).

4. There was a significant increase in total caecal, faecal concentration and daily excretion of DAPA in adult rats fed 10% pectin.

5. Wheat bran did not affect total caecal SCFA, or the relative proportions of the individual SCFA compared to the basal diet, in both adult rats and rats raised entirely on the test diets.
6. The influence of pectin on the caecal SCFA concentration and molar proportions of individual SCFA in the adult rat differed with both 5 and 10% levels, the presence of wheat bran in the diet, and compared to those rats which had been entirely raised on pectin. This was considered to be mainly due to incomplete adaptation, and therefore weaned rats were considered to be more adapted to pectin. Adults fed 5% pectin, and rats weaned onto 10% pectin, gave fermentations which were rich in propionate which may be of importance in lipid metabolism.

7. Weights of caecal-sac and contents were increased with 10% pectin. The muscle-layer thickness was reduced in the proximal colon with pectin, and increased in the distal colon with wheat bran.

8. Raw CRM(X) produced a greater increase in dry stool weight compared to heat-treated CRM(X).

9. Only pectin increased faecal bound water (g/g dry weight faeces) measured in vitro. Supplementation of the diet with wheat bran did not influence faecal bound water.
CHAPTER 1.
INTRODUCTION
1.1. Dietary fibre in health and disease

Epidemiological studies have linked bowel disorders, such as constipation, diverticular disease and cancer of the colon, and also a range of metabolic disorders, to diets deficient in fibre (Cleave & Campbell, 1966; Burkitt & Trowell, 1975; Painter, 1975). As yet, there is no conclusive laboratory evidence as to the aetiology of these 'western diseases', but it is generally believed that they are diet-related. Although fibre was the first to attract attention, it is now realised that the typical western low fibre diet is also lower in starch and higher in salt, sugar, animal proteins and saturated fats compared to the relatively high fibre diets of rural Africans and Asians. It may well be that some other factor promotes disease when total dietary fibre is low. However, dietary fibre influences bowel function, and there is current interest in the therapeutic value of different types of dietary fibre for bowel disorders such as certain cases of constipation, irritable bowel and diverticular disease.

Dietary fibre has a variety of effects along the gastrointestinal tract (Royal College of Physicians, 1980). In the large intestine, these include bacterial fermentation/metabolism, absorption of bacterial metabolites, transit time, intracolonic pressure, flatus production, faecal weight and composition, frequency of defaecation and faecal water content. However, generalisations as to how fibre behaves in the colon
can be misleading, since the extent to which fibre affects colonic function varies with the source of dietary fibre. Although, dietary fibre resists digestion by human enzymes, the rich and metabolically active microflora in the colon can degrade dietary fibre to varying extents. The consequences of this bacterial fermentation on colonic metabolism, faecal weight and also events outside the colon are only now being realised.

1.2. Dietary fibre

Dietary fibre is the non-starch polysaccharides and lignins undigested by the endogenous secretions of the human gastrointestinal tract (Southgate, 1976a; Trowell et al., 1976). Originally, when the term dietary fibre was reintroduced (Trowell, 1972) it was limited to the remnants of plant cell walls which resisted digestion, but the present definition has been expanded to include other fibre sources such as algal polysaccharides, plant gums and mucilages. Although, by any definition, the natural source of dietary fibre is derived from the plant cell walls of fruits, vegetables and cereals.

Analytically, non-starch polysaccharides (NSP) are the main components of dietary fibre and can be divided into cellulose and non-cellulosic polysaccharides (NCP). NCP include hemicelluloses, pectins, mucilages and plant gums such as gum arabic and guar gum (Cummings, 1981a). Lignins are not polysaccharides but complex structures
based on phenylpropane units. Naturally occurring lignins vary in structure and composition and can be covalently linked to hemicelluloses in the plant cell wall (Selvendran, 1987).

The present definition of dietary fibre is oversimple and misleading. It concentrates on only one biological feature, that fibre is "undigested" in the upper gastrointestinal tract. This does not necessarily mean unavailable, since fibre can be broken down by the colonic bacteria and the resultant short-chain fatty acids (SCFA) absorbed (McNeil et al., 1978). Furthermore, other components of the diet, mainly certain forms of natural and processed starch (Englyst et al., 1982), are known to reach the colon in substantial quantities but are not included in the present definition for dietary fibre. However, it is difficult to give a more precise definition as dietary fibre varies widely in its effects along the gastrointestinal tract. Eastwood (1986) suggests that measurements such as glycaemic index [retardation of nutrient absorption in the small intestine (Jenkins et al., 1981)] and the potential water-holding capacity of fibre [water-holding capacity after fermentation (McBurney et al., 1985)] would give a better measure of the biological potential than total dietary fibre content. Despite much debate, it would seem that the general trend is towards a chemical definition for dietary fibre.
In recent years, the analyses of dietary fibre has been greatly improved. The crude fibre method has been abandoned for more sophisticated methods, but is still quoted by manufacturers of animal diets. The Van Soest method (1963) separates fibre into neutral detergent fibre [(NDF) cellulose, hemicellulose and lignin] and acid detergent fibre [(ADF) cellulose and lignin]. The disadvantage of this method is that it does not measure the soluble components of fibre and there is insufficient removal of starch, but an enzymatic method developed by Asp et al. (1983) has attempted to overcome these problems. The Southgate method (1976b) separates fibre into water-soluble and insoluble but has poor starch hydrolysis. This method has been modified by Englyst & Cummings (1984) to exclude the determination of starch and lignins. Thus, depending on the analysis, dietary fibre values can vary (Asp & Johansson, 1984).

The average daily intake of dietary fibre in the U.K. is about 20g and is 5 to 10 times greater in rural Africans and Indians (Bingham & Cummings, 1979). These results are based on the Southgate method, but more recent studies calculate that the NSP intake in the U.K. could be as low as 12g (Bingham et al., 1985). Therefore, the amount of starch entering the large intestine may be as great as the NSP (Bingham, 1987).

The complex group of chemical substances which constitute dietary fibre are usually integrated in the normal diet. Many experiments have studied natural
sources of dietary fibre such as wheat bran and vegetables, but a scientific approach is to study purified and well-defined sources of fibre such as pectin, cellulose and gums. However, the physiological responses demonstrated with purified fibres must be interpreted with caution since, in the whole food diet, the response may be different.

Pectins are of particular interest physiologically since they are effective hypocholesterolaemic agents in humans (Kay et al., 1978) and rats (Judd & Truswell, 1985) and can also reduce glucose absorption from the small intestine (Jenkins et al., 1978). Pectins, or pectic substances are structural components of the plant cell wall and may account for up to 10% of the dry weight of many fruits and vegetables. Pectin functions as an intercellular cementing agents. In the purified form, pectins are used world-wide by the food industry as thickening agents as they are water-soluble and form gels. The main backbone component of pectins is D-galacturonic acids with rhamnose residues inserted at intervals, and sugars such as galactose and arabinose occur in side-chains. Some of the carboxyl groups on the galacturonic acid residues are esterified, usually with methoxyl groups, and less than 50% of the carboxyl groups as methyl esters is regarded as low-methoxyl pectin. The maximum percentage of methyl ester in pure galacturonic acid is 16%, and the 50% ester content is taken as 7% (Judd & Truswell, 1985).
Cereals account for about a third of the daily intake of dietary fibre (Bingham & Cummings, 1979). Wheat bran is one of the most effective dietary fibres at increasing stool weight (Eastwood et al., 1973). Wheat bran is a mixture of cell types, namely the pericarp, aleurone layer and endosperm. The dietary fibre content varies between different sources of bran, and also depends on the extent of milling (Fisher, 1985). The outer pericarp is highly lignified and the aleurone layer is high in protein and lipid.

1.3. Experimental models

Human studies on dietary fibre metabolism and faecal output are complicated by the great variation in response between individuals, and with the difficulty in maintaining humans on strict diets for long periods under controlled conditions. Also, the human caecum is inaccessible to invasive sampling. Therefore, some degree of simplification is required by establishing suitable experimental, animal or in vitro, models. The rat has been widely used since it is easy to handle, relatively inexpensive to maintain, and in-bred lines can keep experimental variability to a minimum. However, the anatomy of the rat's large intestine is different from that of the human. The rat has a capacious caecum and a non-voluminous colon, whereas the human colon is of approximately uniform calibre throughout. Bacterial fermentation occurs mainly in the
right-hand side of the human colon (caecum), and the left hand side is involved with continence. Also, the rat practices coprophagy reingesting 30-65% of its faeces, but this does not appear to affect the fermentation of NDF (Cree et al., 1986). Van Soest et al. (1983) have reported that fibre digestion in humans is more extensive than in the rat, and that the pig is a more suitable model. However, a recent comparative study between man and the rat indicates that the rat experimental model is useful for the prediction of fermentative breakdown and bulking capacity in humans (Nyman & Asp, 1986).

1.4. Dietary fibre and faecal bulking

The daily stool weight is 100-150g for people eating average western diets and 400-500g for Africans (Burkitt et al., 1972). This is a reflection mainly of the fibre content in the diet. High faecal weights are also associated with faster intestinal transit time (Eastwood et al., 1986a). The way in which fibre exerts these effects on colonic function is still not fully understood.

The physical properties of fibre are important when explaining how fibre behaves in the gastrointestinal tract. Water-holding capacity (WHC), the ability of fibre to take up and retain water has been of particular interest in determining the influence different fibres have in the gastrointestinal tract.
Water exists in three phases in relation to fibre (Robertson & Eastwood, 1981a): (1) water which is tightly bound by the hydrophilic polysaccharides of the fibre. This water is unavailable and can only be removed with difficulty, e.g. freeze-drying which leads to an irreversible change in the polymeric structure; (2) interstitial water held in the fibre matrix. This water is available depending on the pore size of the fibre matrix; (3) loose water trapped within the cell wall lumen, which is readily available. WHC can be measured by centrifugation (McConnell et al., 1974), filtration (Robertson & Eastwood, 1981a), column chromatography (Anderson & Eastwood, 1987), and a suction method which measures the ability of fibre to hold onto water against an osmotic pressure (Robertson & Eastwood, 1981b). The suction technique is the most suitable method since it distinguishes between the different phases of water, can be applied to gels, and is more physiological. The amount of trapped water present in fibre will depend on the fibre source, the mode of preparation and the method of measurement. Wheat bran holds 2-6g water per g of bran, whereas fruits, vegetables and gums hold from 8-30g water in each g of fibre (McConnell et al., 1974).

The WHC of those fibres which resist fermentation is believed to be directly related to the biological effect on stool weight (Eastwood, et al., 1983). Coarse bran holds more water in vitro and increases faecal
weight compared to fine bran. However, these WHC methods are unsuitable for assessing the biological effect of the water-soluble fibres on stool bulk, where there is an inverse relationship between WHC and effect on stool weight (Stephen & Cummings, 1979). This is caused by fermentation of these fibres and subsequent loss of WHC. Recently, the suction method has been used to measure the potential water-holding capacity (PWHC) of fibre after a 24 hour in vitro fermentation of fibre with a faecal inoculum (McBurney et al., 1985). This method can give a good prediction of the faecal bulking capacity of dietary fibre.

Faeces are a complex, heterogeneous mixture of bacteria, food residues (undigested and/or partially degraded fibre), soluble ions, organic compounds (protein and fat), endogenous substances (mucus) and water. The average stool is about 75% water, and this is mainly distributed between the bacterial and fibre components of the faeces. A stool fractionation method has analysed the bacterial, fibre and water components of faeces from subjects fed a control diet supplemented with either cabbage or wheat bran (Stephen & Cummings, 1980a). The control diet was based on an average U.K. diet containing about 20g of mixed cell wall polysaccharides. The microbial biomass was about 55% of the dry faecal weight which is considerably more than previous estimates of 30% from microscopic counts. Compared to the control diet, stool weight increased by
127% with wheat bran and by 69% with cabbage. This study demonstrated that fibre can increase stool weight by two mechanisms. First, wheat bran is resistant to fermentation and is available for faecal bulking by its WHC. Secondly, cabbage is extensively fermented and the increase in stool weight could be accounted for by more bacteria. Therefore, fermentable fibres may stimulate bacterial growth and increase microbial cell excretion in faeces. Bacteria are about 80% water and hence an important water holding component of faeces.

Therefore, when studying the faecal bulking properties of dietary fibre it is important to determine the WHC of the fibre, the extent to which the fibre is fermented and, the bacterial biomass which may proliferate.

There are other factors which may affect stool bulk. The production of gas may bulk the faeces. Also, transit time is an important factor determining stool weight, since a faster transit through the gut influences bacterial proliferation (Stephen et al., 1987), the breakdown of fibre (Section 1.6.4), and reduces the time for water absorption.

1.5. Dietary fibre and bacterial fermentation

The caecal fermentation of fibre in some monogastric animals e.g. the horse, pig and rat is widely recognised. It is now accepted that a similar process also occurs in the human large intestine where
the enormous population of bacteria, anaerobic conditions, near neutral pH and moisture are all conducive to fermentation. The major end-products of fermentation are short-chain fatty acids (SCFA) and gases (carbon dioxide, hydrogen and methane). The gases can be absorbed and expired in the breath, and/or excreted as flatus (McKay et al., 1981).

The human large intestine contains about $10^{11}$ bacteria per gram dry weight. The bacterial flora can vary slightly between individuals and around 400 distinct species have been isolated. Over 90% of these bacteria are obligate anaerobes, the rest are facultative anaerobes (oxygen-tolerant) (Moore et al., 1978). The predominant genera are Bacteroides (ca. 25%), Eubacterium (ca. 25%), Bifidobacterium (ca. 12%), Peptostreptococcus (ca. 9%) and Fusobacterium (ca. 8%). Bacteria have also been found attached to the small and large intestinal mucosa in rodents (Abrams, 1983).

Studies on faecal isolates show that many strains of colonic bacteria can ferment plant polysaccharides. Most of these are species of Bacteroides which also have the widest polysaccharide fermenting abilities (Salyers & Leedle, 1983). The polysaccharide-degrading enzymes can be very complex. A recent study on intestinal pectin-degrading bacteria isolated 42 strains of pectinolytic bacteria from human faeces. Of these, two strains of Bacteroides only used pectin and a few related compounds as fermentable substrates (Jensen &
Canale-Parola, 1986). In the large intestine, the major source of fermentable carbohydrates is most probably dietary fibre, but some bacteria are also capable of degrading mucopolysaccharides (Salyers & Leedle, 1983).

1.6. Factors which influence fibre breakdown

1.6.1. Physical and chemical properties

The breakdown of fibre is dependent upon the physical and chemical properties of fibre. Chemically, dietary fibre is complex with many different types of linkages and branching in the polysaccharide. In a recent review Eastwood et al. (1986b) were unable to find any correlation between the chemical composition of a range of dietary fibres and their physiological effects. However, a number of physical properties can influence the breakdown of dietary fibre and its physiological effects in the colon. This includes solubility, water-holding capacity, particle size (insoluble fibres), and cation exchange capacity (Van Soest, 1984).

In general, water-soluble fibre such as pectin (Cummings et al., 1979), gum arabic (McLean Ross et al., 1984) and some hemicellulloses (Slavin et al., 1980) are more readily fermented than the insoluble cellulosic fraction of fibre. When equal doses of fibre from either cabbage or bran were fed to healthy subjects, 10% of the cabbage and 60% of the bran could be recovered in the faeces (Stephen & Cummings, 1980a). The higher
degree of lignification also explains the relative resistance of wheat bran as lignin restricts bacterial access for fermentation (Selvendran et al., 1980).

Particle size is assumed to affect the susceptibility of fibre to fermentation, but results are not consistent especially for faecal dry weights. Heller et al. (1980) found in man that the fermentation of cellulose was more pronounced with fine bran than with coarse bran (not significant), but Ehle et al. (1982) reported a slight increase in the fermentation of cellulose in pigs given coarse wheat bran. Others report that particle size does not affect the fermentation of dietary fibre in the rat (Bjorck et al., 1984; Nyman & Asp, 1985a). However, coarse particle size is the most effective at increasing faecal wet weight (Brodribb & Groves, 1978; Van Soest, 1984; Jenkins et al., 1987).

1.6.2. Food processing and heat treatment

Certain types of food processing and cooking may alter the physiochemical properties of dietary fibre, and hence the digestion of fibre and its influence on faecal bulking (Wyman et al., 1976). Cooking and certain forms of heat treatment cause the matrix structure of vegetables to collapse and consequently affects the ability to hydrate. Pectin in cooked vegetables may be more soluble than those of uncooked vegetables and fruits (Selvendran, 1985). Also,
Maillard products, polymers which are formed by a non-enzymatic reaction between reducing sugars and amino-acids, peptides or proteins, may be produced during cooking or storage (Hodge, 1953; Ellis, 1959). These compounds reach the colon intact, but very little is known about the colonic metabolism of Maillard products. However, early products in the reaction such as fructose-L-tryptophan (Tanaka et al., 1976), and intermediates in the reaction (Salyers & Leedle, 1983) may be metabolised by colonic bacteria.

The extraction process to obtain purified sources of fibre from the plant cell walls alters the physical and chemical properties of the original fibre. For example, purified cellulose is more resistant than normal cellulose to fermentation (Van Soest, 1978; Ehle et al., 1982).

1.6.3. Level of fibre and the presence of other dietary components

The amount of fibre in the diet may also be expected to affect fibre breakdown. Increased fibre levels have been reported to decrease fibre digestibility (Garrison et al., 1978), but Nyman and Asp (1985) report that the level of fibre does not influence the breakdown of either wheat bran or pectin in the rat.

The breakdown of a fibre may be affected by the presence of another fibre component in the diet (Wise et al., 1986; Topping et al., 1985). Components in the
diet such as protein, starch, and fat may escape digestion in the upper gut and affect the metabolism of dietary fibre (Brockett & Tannock, 1982).

1.6.4. Host factors

There is considerable variation in fibre breakdown between individuals fed identical diets, as well as individual variation from week to week. This variation is, in part, related to intestinal transit time. The mouth-to-anus transit time, measured by continuous and intermittent markers, averages 48 to 72 hours in normal subjects in the U.K., and 40 to 60 hours of this time is spent in the large intestine (Cummings, 1984).

The retention of digesta in the large intestine is an important factor determining the extent of dietary fibre fermentation. If transit time is too fast, below 50 hours in humans, then fibre digestion is reduced (Cummings et al., 1981). Similarly, surgical removal of the rat caecum decreased transit time and reduced fibre digestibility (Lee & Grace, 1980), or increased the amount of gum arabic in colonic contents (McLean Ross et al., 1984).

Another host factor which could influence fibre metabolism is the type of colonic bacterial flora. The species composition of the colonic bacteria is believed to be stable and unaffected by diet (Savage, 1983), but a recent study has reported a change in the faecal flora of a human volunteer following addition of gum arabic to
the diet (Wyatt et al., 1986). Changes in a microbial population have been observed in model human colons when the nutrient supply is changed (Miller & Wolin, 1981), and when human faecal slurries are incubated with different dietary fibres (Slade et al., 1987).

1.6.5. Previous diet and duration of experiment

When the diet is changed to a high fibre regime subjects will require a period of time to equilibrate or adapt to the dietary change. Adaptation may depend on a number of factors but previous diet and age or liveweight of the subjects may be important.

There are a number of reports which demonstrate that prolongation of the duration of a feeding trial does not alter the degree of breakdown of fibre. There were no differences in the fermentation of wheat bran when humans were fed for 6 or 3 weeks (Cummings, 1982). A four day adaptation period is believed to be adequate for evaluating the fermentability of pectin or wheat bran in rats (Nyman & Asp, 1985). These studies determined fibre breakdown by measuring the residual fibre in the faeces. However, a study which measured caecal and faecal SCFA in rats fed wheat bran or gum arabic, has concluded that 4 weeks is sufficient for evaluating stool weight and related analyses (measurements before 4 weeks were not determined) and that a duration of at least 8 to 12 weeks is
needed for studying metabolic events (Walter et al., 1986).

1.7. **Short-chain fatty acids**

Short-chain fatty acids (SCFA) are intermediate and end-products of anaerobic carbohydrate fermentation. These are the major anions in colonic contents and found in concentrations about 100-200 mmol l\(^{-1}\). The predominant SCFA are acetate, propionate and butyrate which are generally found in ratios 60:25:15 respectively (Cummings, 1981b). Isovalerate, isobutyrate and valerate can also be detected in small quantities.

Hellendoorn (1978) believed that SCFA influenced faecal weight, but it is now known that SCFA are rapidly absorbed from the human large intestine (McNeil et al., 1978). The absorption of SCFA is accompanied by a net sodium and water absorption (Roediger & Moore, 1981) and by an accumulation of bicarbonate in the lumen. Bicarbonate is important for maintaining the luminal pH for optimal microbial activity.

The fate of absorbed SCFA has been investigated mainly in animals. SCFA provide the ruminant with about 70% energy (Smith & Bryant, 1979). The energy contribution in humans is unknown, but estimates are about 2 to 7% for people on low dietary fibre intakes in the U.K. and U.S.A. (Cummings, 1981a). However, in communities where dietary fibre intakes are higher then
a significant amount of energy may be gained from the absorption of SCFA. Acetate, propionate and butyrate are metabolised in the liver but only acetate reaches the peripheral circulation in significant amounts (Pomare et al., 1985). Acetate would seem to contribute to energy production only (Snoswell et al., 1982) whereas, propionate may lower plasma cholesterol (Chen et al., 1984) and butyrate appears to be the preferred substrate for isolated colonocytes (Roediger, 1982). In addition, butyrate may have anti-neoplastic properties since it reduces the growth of human large bowel cancer cells (Kim et al., 1982; Kruh, 1982). Also, SCFA may stimulate colonic epithelial production (Sakata & Yajima, 1984).

It has been estimated that each day 30g of carbohydrate is fermented in the human colon to yield 300mmol SCFA (Cummings & Branch, 1986), of which only 10-20% is excreted in the faeces (Meijer-Severs & Santen, 1987). The influence of diet on SCFA production in humans is not clear. However, numerous studies on the caecal SCFA in the pig and rat indicate that dietary fibre influences the amount of caecal SCFA and the molar proportions of individual SCFA (Thomsen et al., 1984; Storer et al., 1983; Demigne & Remesy, 1985, Topping et al., 1985; Walter et al., 1986).
1.8. Colonic structure

In animal experiments, the consumption of high fibre diets can cause an increase in the size and weight of the caecum and colon. The effect varies according to the amount and type of fibre (Elsenhans et al., 1981), period of consumption, and other components in the diet like the type of protein (Southon et al., 1987). In general, the water-soluble fibres produce a greater influence on tissue weight in the caecum, and water-insoluble fibres exert a greater influence in the colon.

In particular, the effect of dietary fibre on the gut mucosa has been examined. The consequence of adding 20% glucomannan (water-soluble) or 20% cellulose (water-insoluble) to a rat diet on the DNA, RNA and protein of the caecal and colonic mucosa has demonstrated that the caecal enlargement caused by glucomannan was due to an increase in both number and size of mucosal cells. The colonic enlargement caused by cellulose resulted from an increase in the number of mucosal cells (hyperplasia) (Konishi et al., 1984). The effect of dietary wheat bran, pectin, guar gum and oat bran on the mucosa of the caecum, proximal and distal colon have been studied (Jacobs & Schneeman, 1981; Jacobs & Lupton, 1984). Wheat bran produced a greater hyperplastic response in the distal colon, whereas guar and pectin produced a greater effect proximally. The mechanism of cell hyperplasia were different with different fibres. Oat bran had no overall effect on the mucosa. Wheat bran
increased the colon muscle-thickness, but measurements for the other fibres were not available.

The mechanism by which fibre causes mucosal proliferation is still unclear but several factors may be involved. Fermentation appears to be important: soluble fibres are metabolised maximally in the caecum and proximal colon where mucosal proliferation tends to occur. SCFA accelerate epithelial cell proliferation, and bulk (in the form of kaolin) increases colonic tissue weight, but the actions of these factors are independent of each other (Sakata, 1986). The acidification of the luminal contents following fermentation may be important since there is greater cell proliferation at lower pH (Lupton et al., 1985). Other factors include hormonal (Southon et al., 1987), cation binding, and bile acids.

The effect of dietary fibre on colonic muscle has received little attention. The increase in the weight of the colon in rats fed up to 66% kaolin was due to increased muscle-thickness (Dowling et al., 1967). Thickening of the colonic muscle also occurs after feeding rats 20% wheat bran for two weeks (Jacobs & Schneeman, 1981). Pectin (18%) increases the muscle-thickness in the small intestine, and it was assumed that the increased weight of the colon was due to a similar effect (Brown et al., 1979). This has not been supported by histological studies. Therefore, it is assumed that bulk plays a role in determining muscle
thicknes, but other mechanisms may be involved such as hormonal or calcium binding (Jacobs, 1985).

1.9. **Aims**

1) To investigate the effects of two contrasting fibre sources, wheat bran (insoluble) and high-methoxyl pectin (soluble), on colonic metabolism (weight of caecal contents and sac, and caecal and faecal SCFA), stool weight and colonic mucosa and muscle thickness in the male rat.

2. To estimate colonic bacterial biomass and numbers by measuring diaminopimelic acid (DAPA) and using a plate-count method respectively.

3. To measure the water-holding capacity of faecal and caecal material with an *in vitro* method, using dialysis membranes and polyethylene glycol to produce an osmotic gradient.

4. To study the influence of heat-treated fibre on colonic metabolism and faecal weight.
2.1. Animals and diets

2.1.1. Test diets

The basal diet (Special Diets Services, Witham, Essex.) was composed of protein, fat and carbohydrate of plant origin (Table 2.1). The two dietary fibres studied were high-methoxyl Pectin-USP derived from citrus peel [(Table 2.2), H P Bulmer, limited, Hereford] and coarse Canadian Red Spring Wheat bran [(Table 2.3), Chancelot Mills, Edinburgh].

2.1.2. Stock diet

The stock diet, CRM(X), was an expanded form of pellet diet formulated for small animals by Labsure, Croydon (Tables 2.4 and 2.5).

2.1.3. Formulation of test diets

The test diets were formulated by supplementing the basal diet with a known amount of fibre. Unsupplemented basal was used as a control and the only variable in the other test diets was the fibre source.

1) Basal
2) 10% wheat bran (100g kg⁻¹)
3) 10% pectin (100g kg⁻¹)
4) 5% pectin + 5% wheat bran (50g kg⁻¹ each fibre)
5) 5% pectin (50g kg⁻¹)
6) 20% wheat bran (200g kg⁻¹)

The composition and energy values for test diets (1 to 5) and CRM(X) are given in Tables 2.5 and 2.6 respectively.
Table 2.1. Levels of constituents in the basal diet.

<table>
<thead>
<tr>
<th>Dietary constituent</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya concentrate</td>
<td>20</td>
</tr>
<tr>
<td>Soya oil</td>
<td>3</td>
</tr>
<tr>
<td>Cornflour</td>
<td>72</td>
</tr>
<tr>
<td>Premix *</td>
<td>5</td>
</tr>
</tbody>
</table>

* Nutritionally complete vitamin and mineral premix. Information supplied by Special Diets Services, Witham, Essex.

Table 2.2. Molecular weight, electrolyte concentration, viscosity and degree of esterification of pectin-USP.

<table>
<thead>
<tr>
<th>Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>4.5 - 5.5 (dl g⁻¹)</td>
</tr>
<tr>
<td>Electrolyte concentration</td>
<td>0.1 - 1.5 (%)</td>
</tr>
<tr>
<td>Viscosity *</td>
<td>200 - 400 (m Pa s)</td>
</tr>
<tr>
<td>Degree of Esterification</td>
<td>68 - 72 (%)</td>
</tr>
</tbody>
</table>

* Viscosity was measured in centipoise units for 2% solution at 25°C. Analyses were carried out by H P Bulmer Limited, Hereford.
Table 2.3. Analyses of Canadian Red Spring Wheat bran (coarse particle size).

<table>
<thead>
<tr>
<th>Composition</th>
<th>% dry matter</th>
<th>Sieving tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aperture (mm)</td>
</tr>
<tr>
<td>Starch</td>
<td>17.5</td>
<td>3.55</td>
</tr>
<tr>
<td>Protein</td>
<td>14.8</td>
<td>2.80</td>
</tr>
<tr>
<td>Fat</td>
<td>6.4</td>
<td>1.50</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>22.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Cellulose</td>
<td>9.7</td>
<td>0.50</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td>0.44</td>
<td></td>
</tr>
</tbody>
</table>

Data from Anderson & Eastwood (1987).

Table 2.4. Dietary fibre content of Labsure CRM

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% of total*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemicellulose</td>
<td>59.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>27.6</td>
</tr>
<tr>
<td>Lignin</td>
<td>8.3</td>
</tr>
<tr>
<td>Pectin</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Total dietary fibre is 159 g kg dry weight⁻¹
Data from Wise & Gilburt (1980).
(Major constituents of CRM(X) (% crude fibre): wheat (1.2), wheat feed (1.3), soya extract (0.57), maize (0.38). Information supplied by Labsure, Croydon.
Table 2.5. Composition of CRM(X), basal diet, and basal supplemented with 5% pectin, 10% wheat bran, 10% pectin, and with 5% pectin mixed with 5% wheat bran.

<table>
<thead>
<tr>
<th>Dietary Constituent (%)</th>
<th>CRM(X)</th>
<th>Basal diet</th>
<th>10% bran</th>
<th>10% pectin</th>
<th>5% bran + 5% pectin</th>
<th>5% pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>18.3</td>
<td>14.8</td>
<td>12.3</td>
<td>14.3</td>
<td>10.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.9</td>
<td>2.5</td>
<td>2.1</td>
<td>2.5</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>56.3</td>
<td>68.0</td>
<td>70.2</td>
<td>68.4</td>
<td>72.6</td>
<td>68.1</td>
</tr>
</tbody>
</table>

Analyses were carried out by Special Diets Services Witham, Essex. Analyses on CRM(X) were provided by Labsure, Croydon.

Table 2.6. Energy Values for CRM(X), basal diet, and basal supplemented with 10% wheat bran, 10% pectin, and 5% wheat bran mixed with 5% pectin.

<table>
<thead>
<tr>
<th>Energy</th>
<th>CRM(X)</th>
<th>Basal diet</th>
<th>10% bran</th>
<th>10% pectin</th>
<th>5% bran + 5% pectin</th>
<th>5% pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross</td>
<td>_</td>
<td>15.4</td>
<td>14.7</td>
<td>15.0</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>Digestible</td>
<td>_</td>
<td>13.9</td>
<td>13.2</td>
<td>13.6</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Metabolisable</td>
<td>12.2</td>
<td>12.5</td>
<td>11.9</td>
<td>12.2</td>
<td>11.9</td>
<td></td>
</tr>
</tbody>
</table>

Analyses were carried out by Special Diets Services Witham, Essex. Analysis on CRM(X) was provided by Labsure, Croydon.
2.1.4. **Animals**

Albino Wistar rats were bred in the Animal Unit, Western General Hospital, Edinburgh. Prior to experimentation, adult rats were maintained on CRM(X). Male rats were used in all experiments.

2.1.5. **Housing**

Animals were housed in solid-bottomed cages with white wood shavings for bedding. The room was regulated to a 12 h light-12 h dark schedule, maintained at 20-21°C and noise level was kept to a minimum. Animals were kept within the facilities of the Animal Unit, Western General Hospital, Edinburgh.

2.1.6. **Feeding regime**

Diets were prepared at 10 a.m. on a daily basis. Dry weight of diets were recorded and mixed with a known amount of tap water to form a stiff paste and presented in stainless-steel bowls. Food and water were provided *ad libitum*.

2.1.7. **Health maintenance**

General health was assessed regularly and increases in body weight were used as an indication of good health. Teeth were inspected regularly and over-long teeth were clipped.
2.1.8. Post mortem

Animals were placed in a bell-jar, anaesthetised with diethyl ether and then killed by cervical dislocation. In order to eliminate the effects of diurnal variation, animals were sacrificed within 45 minutes of 10 a.m. for all experiments.

2.2. Food intake and body weight gain

2.2.1. Body weight

Individual body weights were recorded at the beginning of the experiment, and thereafter, at the same time each week until final body weights were taken before animals were sacrificed.

2.2.2. Food Consumption

Assessment of daily food intake was determined from left-over food and calculated on a dry weight basis.

2.3. Sample collection

2.3.1. Faeces

Rats were transferred to individual, gridded cages seven days before the end of the experiment. Total faecal output of each animal was collected in sterile vials during the 3 final consecutive 24h periods, weighed and stored at -20°C.
2.3.2. **Caecal contents**

The caecum was isolated at the ileo-caecal valve and the beginning of the transverse colon, dissected out, transferred to a pre-weighed vial and weighed. The caecum was opened and the caecal contents scraped into a pre-weighed vial, weighed and stored at -20°C.

2.3.3. **Caecal-sac**

The empty caecal-sac was washed out with 0.9% NaCl, blotted dry with filter paper and weighed in a vial.

2.4. **Freeze-drying samples**

Samples were frozen at -20°C for at least 24 hours before freeze-drying. The lid of the container was replaced by a fine gauze and secured with a rubber band. Samples were processed for 48 hours in the freeze-drier and stored at -20°C.

2.5. **Short-chain fatty acids**

A modification of the method described by Spiller et al. (1980) was used to determine the SCFA in caecal and faecal material.

2.5.1. **Preparation of samples**

Faecal and caecal material were collected as described in Section 2.3., adjusted to pH9 with dilute NaOH, frozen at -20°C, freeze-dried (Section 2.4) and ground to fine particle size with a pestle and mortar.
2.5.2. Extraction

Samples, about 100mg, were accurately weighed. Orthophosphoric acid (0.1cm³), distilled water (0.8cm³) and the internal standard, 4-methyl valeric acid (50mm³), were added to the weighed sample. SCFA were extracted 3 times with 3cm³ diethyl ether and extracts were mixed together.

2.5.3. Separation

Separation of SCFA was achieved by gas-liquid chromatography. Extracts of 3mm³ were injected onto a SP2250 filled glass column. The carrier gas, nitrogen, was maintained at a flow of 60cm³ per minute. The temperature was programmed between 80°C and 150°C at 16°C per minute. A standard curve was made from serial dilutions, 0.01 to 0.3cm³, of standard SCFA and a constant amount of internal standard (50mm³).

2.5.4. Measurement

Sample peaks for acetate, propionate, butyrate, isobutyrate, isovalerate and valerate were recorded on a pen-recorder. Peak height ratios were calculated from individual peaks and the internal standard peak height. The concentrations of SCFA were determined from the standard curve.
2.6. Statistical treatment

One-way analysis of variance was used when comparing more than two means, after logarithmic transformation to stabilise the variance if necessary. Means were compared using the t-test and differences were considered significant at $p<0.05$. The unpaired t-test was used for comparing two means and, if the variance was still not stable after logarithmic transformation, the Mann Whitney U-test was used. Differences between means were considered significant at $p<0.05$, $<0.01$, $<0.001$ for the unpaired t-test and at $p<0.05$ for the Mann Whitney U-test. Correlation coefficients were calculated using an Amstat statistical package.
CHAPTER 3.

MEASUREMENT OF BACTERIAL NUMBERS AND DIAMINOPIMELIC ACID
3.1. **Aim**

Diaminopimelic acid (DAPA) is a component of the bacterial cell wall. A recent study in this laboratory estimated bacterial biomass, by measuring DAPA, in the colonic contents of rats fed wheat bran or gum arabic, and reported an increase in caecal and faecal DAPA in rats fed the latter diet (Walter et al., 1986). However, DAPA is not present in all gut bacteria, and also varies in the content between species. Therefore, it could not be determined whether an increase in DAPA content was due to an overall increase in bacterial numbers, or due to an increase in DAPA-rich bacteria. Therefore, in this present study, in addition to measuring the DAPA content of colonic material from rats fed wheat bran or pectin, the number of bacteria was also estimated using a plate-count technique (Veilleux & Rowland, 1981).

3.2. **Materials and methods**

3.2.1. **Animals and diets**

Male rats, aged 6 weeks old, were divided into 5 groups of 5. Four groups were allocated to a test diet and one group was continued on CRX. The 4 test diets were basal, or basal supplemented with 10% wheat bran, 10% pectin, and 5% pectin mixed with 5% wheat bran (Section 2.1.3). Animals were fed each diet for 28 days. Animals were housed in groups of 5 and maintained as described in Section 2.1.
3.2.2. **Enumeration of caecal aerobic and anaerobic bacteria**

3.2.2 a. **Aseptic technique**

Aseptic technique was maintained throughout the experiment. Glass equipment was sterilised in the pressure-cooker for 30 minutes at 120 p.s.i. Metal instruments were wrapped in kraft-paper and sterilised in a hot-air oven (with fan circulation of air) for 1 hour at 160°C.

3.2.2 b. **Collection of caecal contents**

The excised caecum (Section 2.3.2) was immediately transferred to a sterile vial, which had been previously gassed with O₂-free-N₂, and the lid was then firmly closed.

3.2.2 c. **Maintenance of an anaerobic environment**

A flexible glove-bag (Mackay and Lynn, Edinburgh) was used to maintain a low oxygen environment. A small port allows the bag to be filled with gas, a larger port gives access to the inside, and there are two glove ports. The necessary vials and equipment were placed inside the bag, it was filled and flushed 3 times with O₂-free-N₂ and then the access port was tightly sealed with metal clips.
3.2.2 d. Dilution of caecal contents

Reinforced clostridial medium [RCM (Oxoid)] was used as a diluent and prepared as stated by the manufacturers. The diluent was dispensed in 9cm³ amounts into glass universals, with metal caps and rubber seals, and then sterilised in the pressure cooker for 15 minutes at 120 p.s.i. The caps were loosened and the diluents were then placed in a shallow boiling water-bath for 10 minutes to pre-reduce the medium. The caps were then firmly sealed and dilutions were carried out in the glove-bag.

About 1g of mixed caecal contents was added to a pre-weighed vial of diluent, sealed and then mixed with a vortex-type mixer for 10 minutes to give a 10¹ dilution. The 10¹ dilution was re-weighed to find the accurate weight of the added sample. 1cm³ of the 10¹ dilution was pipetted into 9cm³ diluent to give a 10² dilution. Subsequent 10-fold dilutions were made up to 10⁶.

3.2.2 e. Spreading agar plates

Freshly made blood agar, dispensed into sterile, disposable petri-dishes, were supplied by the Central Microbiology Department, Western General Hospital, Edinburgh. Six aliquots of 20mm³ from each dilution (10⁵ to 10⁶) were inoculated onto six agar plates, using an autoclavable, repetitive pipette (BDH), and spread evenly over the surface with a glass spreader.
3.2.2 f. Anaerobic incubation

Triplicate petri-dishes from each dilution, an active catalyst, and an agar plate inoculated with an O_2-sensitive organism (Pseudomonas spp.) were placed in an anaerobic jar (Don Whitley Scientific Limited, Shipley). The jar was sealed, evacuated with a vacuum pump, refilled with a mixture of 3% (v/v) H_2, 10% (v/v) CO_2, and 87% (v/v) N_2 and incubated at 37°C for 72 hours.

3.2.2 g. Aerobic incubation

Triplicate petri-dishes from each dilution were incubated at 37°C for 48 hours.

3.2.2 h. Enumeration of bacteria

Dilutions which gave 50 to 150 colonies per plate were chosen and colony-forming units (C.F.U.) were counted. The number of bacteria per gramme wet weight of caecal contents was determined and expressed as log_{10} C.F.U.g dry weight^{-1}.

3.2.3. Determination of caecal and faecal DAPA

Caecal and faecal material were collected as described in Section 2.3. Samples were freeze-dried and then ground to a fine particle size with a pestle and mortar. The amount of 2-6-diaminopimelic acid (DAPA) in freeze-dried material was determined according to Czerkawski (1974) with the following modifications:
For the hydrolysis procedure, culture tubes with screw-caps (BDH) were placed in a hot-block at 105°C. Amberlite CG-120 (Na), mesh size 100-120, supplied by Fluka (U.K. agents, Flurochem Limited, Glossop, Derbyshire), was used as the ion-exchange resin.
3.3. RESULTS

3.3.1. Caecal bacterial numbers

Table 3.1. shows the caecal aerobic and anaerobic counts. The anaerobic count also includes the facultative anaerobes (aerobe count), and is therefore a total count of the number of viable bacteria. The concentration of anaerobes and the total number of anaerobes per caecum were significantly increased with 10% pectin (p<0.05). The number of anaerobes for the 10% bran diet was not significantly different from the basal diet. The 10% bran and the 10% pectin diets significantly increased the concentration of aerobes and decreased the anaerobe:aerobe ratio compared to the basal diet.

3.3.2. Caecal and faecal DAPA

The results for caecal DAPA are shown in Table 3.2. The DAPA concentration and total DAPA per caecum significantly increased with the pectin/bran mixed diet. The increase in DAPA concentration observed with 10% pectin was not significant, but the increased dry weight of caecal contents with this diet (Section 4.3.2., Fig. 4.3), significantly increased the total DAPA per caecum (p<0.05). Caecal DAPA from rats fed CRM(X) was similar to the 10% bran diet. DAPA significantly correlated with bacterial numbers (r=0.45, p<0.05).

The pectin-containing diets significantly increased the total daily excretion of faecal DAPA (Table 3.3).
Table 3.1. Concentration of aerobic and anaerobic bacteria, total anaerobes per caecum and anaerobe:aerobe ratio in caecal contents from adult rats fed CRK(X), basal, and basal supplemented with 10% wheat bran or 10% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Aerobes (Log$_{10}$ C.F.U. g dry weight$^{-1}$)</th>
<th>Anaerobes $^{a}$</th>
<th>Total anaerobes caecum$^{-1}$</th>
<th>Anaerobe:Aerobe$^{*}$ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8.32$^{a}$</td>
<td>10.49$^{a}$</td>
<td>10.25$^{a}$</td>
<td>148 : 1</td>
</tr>
<tr>
<td>10% bran</td>
<td>9.67$^{b}$</td>
<td>10.51$^{a}$</td>
<td>10.33$^{a}$</td>
<td>7 : 1</td>
</tr>
<tr>
<td>10% pectin</td>
<td>9.30$^{c}$</td>
<td>11.00$^{a}$</td>
<td>10.97$^{a}$</td>
<td>50 : 1</td>
</tr>
<tr>
<td>SEM (df)</td>
<td>0.11 (12)</td>
<td>0.08 (12)</td>
<td>0.08 (12)</td>
<td></td>
</tr>
<tr>
<td>CRK(X)</td>
<td>9.11 (0.09)</td>
<td>10.45 (0.08)</td>
<td>10.42 (0.07)</td>
<td>22 : 1</td>
</tr>
</tbody>
</table>

* Anaerobe:aerobe ratio was calculated before log$_{10}$ transformation.
Means in vertical columns not sharing a common superscript differed significantly (t-test); p < 0.05.
CRK(X) results expressed as mean (SEM in parentheses).
Table 3.2. Caecal DAPA concentration and total DAPA per caecum in adult rats fed CRM(X), basal, and basal supplemented with either 10% wheat bran or 10% pectin, and 5% wheat bran mixed with 5% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>(n)</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>(5)</td>
<td>3.5 ^a</td>
<td>0.6</td>
<td>2.0 ^a</td>
</tr>
<tr>
<td>10% bran</td>
<td>(5)</td>
<td>2.6 ^a</td>
<td>0.6</td>
<td>1.8 ^a</td>
</tr>
<tr>
<td>10% pectin</td>
<td>(4)</td>
<td>4.4 ^b</td>
<td>0.7</td>
<td>4.4 ^b</td>
</tr>
<tr>
<td>5% pectin/5% bran</td>
<td>(5)</td>
<td>6.0 ^b</td>
<td>0.6</td>
<td>4.6 ^b</td>
</tr>
<tr>
<td>CRM(X)</td>
<td>(5)</td>
<td>2.5</td>
<td>0.1</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

Means in vertical columns not sharing a common superscript differed significantly (t-test); p < 0.05. CRM(X) is shown as mean ± SEM.
Table 3.3. Faecal DAPA concentration and total DAPA excreted per day in adult rats fed CRM(X), basal, and basal supplemented with either 10% wheat bran or 10% pectin, and 5% wheat bran mixed with 5% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>(n)</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5</td>
<td>4.7 A</td>
<td>0.06</td>
<td>5.7 A</td>
<td>0.8</td>
</tr>
<tr>
<td>10% bran</td>
<td>4</td>
<td>3.4 C</td>
<td>0.07</td>
<td>7.2 AC</td>
<td>0.7</td>
</tr>
<tr>
<td>10% pectin</td>
<td>5</td>
<td>8.1 b</td>
<td>0.06</td>
<td>11.8 b</td>
<td>0.7</td>
</tr>
<tr>
<td>5% pectin/5% bran</td>
<td>6</td>
<td>5.4 AC</td>
<td>0.05</td>
<td>8.5 c</td>
<td>0.7</td>
</tr>
<tr>
<td>CRM(X)</td>
<td>5</td>
<td>3.2 AB</td>
<td>0.10</td>
<td>14.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

** SEM of log-transformed data. Means in vertical columns not sharing a common superscript differed significantly (t-test); p < 0.05
CHAPTER 4.

EFFECTS OF FEEDING ADULT RATS SINGLE AND MIXED SUPPLEMENTS OF WHEAT BRAN AND PECTIN ON:

A) COLONIC METABOLISM AND FAECAL OUTPUT

B) COLONIC WALL STRUCTURE
4.1. **Aim**

The aim of this study was to investigate the colonic metabolism of wheat bran and pectin, as single and mixed supplements, in the adult rat. Also, the effect of these fibres on the colonic muscle and mucosal thickness was examined.

4.2. **Materials and methods**

4.2.1. **Animals and diets**

Male rats, aged 6 weeks old and which had been previously maintained on the stock diet, CRM(X), were divided into 7 groups of 5 and housed in groups of 5. Five groups were fed test diets for 28 days, and 2 groups were continued on CRM(X), one group for 4 days and the other for 28 days. Test diets were basal, or basal supplemented with 10% wheat bran, 10% pectin, 5% wheat bran mixed with 5% pectin, and 5% pectin. Diets and animal procedures are described in Section 2.1.

Procedures for sample collection and analysis of SCFA are described in Sections 2.3 and 2.5, respectively.

4.2.2. **Histology**

The large intestine was divided into two segments, the caecum and the colon. The colon was then further divided into two halves, the lengths of which were determined by holding the colon in a hanging position.
Colonic segments were rinsed in cold 0.9% NaCl. Lengths of 1.5cm were taken from the two anatomic sites, proximal and distal colon, and fixed in 10% buffered formaldehyde solution. Samples were embedded in paraffin wax and transverse sections were cut. Well-orientated sections were stained with haematoxylin and eosin. The sections were coded so that the origin of the section was unknown to the observer. The mean of six observations of total muscle and mucosal thickness was calculated for each section using a light microscope equipped with a calibrated micrometer eyepiece.
4.3. RESULTS

4.3.1. Feed intake and body weight gain

The growth curve for rats fed 10% bran was similar to those fed basal diet (Fig. 4.1). The reduction in the energy density of this diet (Table 2.6) did not affect body weight gain, as animals increased food intake and consequently reduced the food conversion efficiency (Table 4.1).

Diets containing pectin significantly reduced body weight gain and the food conversion efficiencies (Table 4.1). The reduced feed intake in the first week of the study was partly responsible for these results (Fig. 4.2). Moreover, the energy densities of supplemented diets were reduced (Table 2.6) so, despite the similar feed intakes with 5% pectin and the basal diet, 5% pectin significantly reduced body weight gain (p<0.05).

The differences between the growth curve (Fig. 4.1), body weight gain, feed intake and food conversion efficiency (Table 4.1) for rats fed pectin/bran mixed diet were reduced compared to the basal diet and 10% bran diet. The results for rats fed CRM(X) were similar to the basal diet.
Fig. 4.1. Growth curves for adult rats fed CRM(X), basal, and basal supplemented with 10% wheat bran, 10% pectin, 5% wheat bran mixed with 5% pectin, and 5% pectin.
Table 4.1. Growth and food conversion efficiency for adult rats fed CRM(X), basal, or basal supplemented with 5% pectin, 10% of either wheat bran or pectin, and with 5% of wheat bran mixed with 5% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Basal</th>
<th>Bran</th>
<th>Pectin</th>
<th>Pectin/bran</th>
<th>Pectin</th>
<th>CRM(X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original wt. (g)</td>
<td>162^a</td>
<td>172^a</td>
<td>172^a</td>
<td>236^b</td>
<td>203^c</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(9)</td>
<td>(9)</td>
<td>(1)</td>
<td>(3)</td>
<td>(9)</td>
</tr>
<tr>
<td>Weight gain (g 28 days^-1 rat^-1)</td>
<td>157^a</td>
<td>138^ac</td>
<td>102^a</td>
<td>87^b</td>
<td>124^c</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(8)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(14)</td>
</tr>
<tr>
<td>Food intake (g 28 days^-1 rat^-1)</td>
<td>673</td>
<td>698</td>
<td>604</td>
<td>523</td>
<td>671</td>
<td>661</td>
</tr>
<tr>
<td>Food conversion efficiency ++</td>
<td>0.23</td>
<td>0.20</td>
<td>0.17</td>
<td>0.17</td>
<td>0.18</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Data are shown as means of 5 rats (SEM in parentheses) or * six rats. Data (except CRM(X)) were analysed using one-way analysis of variance and means within the same horizontal row not sharing a common superscript differed significantly (t-test; p < 0.05). ++ is expressed as g weight gain g food consumed^-1.
Fig. 4.2. Weight gain in relation to food intake in rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin.
4.3.2. **Weight of caecal contents**

Final body weights were different between the test diets therefore, weight of wet and dry caecal contents were related to final body weight (Fig. 4.3). The wet weight of caecal contents was significantly increased with all the pectin-supplemented diets, of which 10% pectin resulted in the greatest increase \( p<0.05 \). However, only the 10% pectin diet significantly increased the dry weight \( P<0.05 \). Wet and dry weights of caecal contents for rats fed 10% bran, were not significantly different from the basal diet, but the wet weight was significantly reduced \( P<0.05 \) compared to the pectin-supplemented diets. The wet weight of caecal contents was intermediate for rats fed the pectin/bran mixed diet compared to these fibres fed separately at the 10% level. The dry weight for pectin/bran mixed diet was closer to the 10% bran group than to the 10% pectin group. The wet weight of caecal contents decreased when rats were fed CRM(X) for 4 weeks compared to the pre-trial group.

4.3.3. **Caecal-sac weight**

Caecal-sac weight was related to final body weight and results are shown in Fig. 4.4. Pectin at 5% and 10% increments similarly increased caecal-sac weight. 10% bran significantly decreased the caecal-sac weight \( p<0.05 \). The pectin/bran mixed diet resulted in the caecal-sac weight being closer to 10% bran than to 10%
Fig. 4.3. Wet and dry weights of caecal contents in relation to body weight from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin.

Each bar represents mean ± SEM for 5 or +6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B, C) were significantly different (t-test); p<0.05.
Fig. 4.4. Caecal-sac weight in relation to body weight from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin.

Each bar represents mean ± SEM for 5 or ++6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B, C) were significantly different (t-test); p<0.05.
pectin. In parallel to the data on the weight of wet caecal contents, the caecal-sac weight was decreased for CRM(X) when compared to the pre-trial group.

4.3.4. Stool weight

All fibre supplements, except the 5% pectin diet, increased the daily dry faecal output (Fig. 4.5) and the dry faecal output when expressed per 100 g food intake (Fig. 4.6). In relation to the basal diet, 10% bran produced the greatest increase in faecal weight (89%) in relation to food intake. The high faecal output with CRM(X) is discussed in Section 5.3.4.

4.3.5. Short-chain fatty acids

The major SCFA in caecal and faecal material were acetate, propionate and butyrate. Isovalerate, valerate and isobutyrate were either undetected in some samples or contributed to less than 5% of the total SCFA.

4.3.5(a). Total caecal concentration

Total SCFA concentration in caecal and faecal material are shown in (Fig. 4.7). In comparison to the basal diet, the total caecal SCFA concentration were reduced with both the 10% bran and the 10% pectin diets, and unaffected with the 5% pectin and the pectin/bran mixed diet. Increasing the amount of pectin from 5 to 10% caused a significant decrease in the total caecal SCFA concentration (p<0.05). Total concentration of
Fig. 4.5. Daily faecal weight from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or 6+ rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B, C, D) were significantly different (t-test); p<0.05.
Fig. 4.6. Faecal weight expressed per 100g food intake from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin.

Each bar represents mean ± SEM for 5 or 6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B, C, D) were significantly different (t-test); p<0.05.
Fig. 4.7. Total caecal and faecal SCFA concentration from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin.

Each bar represents mean ± SEM for 5 or ≥6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (a, b, c) were significantly different (t-test); p<0.05.
caecal SCFA were similar in the 10% bran and the 10% pectin groups, but this was significantly increased \( p<0.05 \) when pectin and bran were fed as a mixture. The diets supplemented with 5% pectin, either as a single or mixed fibre fraction, yielded the highest total caecal SCFA of the test diets.

4.3.5(b). Total faecal concentration

The total faecal SCFA concentration significantly increased with both the 5 and 10% pectin diets and the pectin/bran mixed diet when compared to the basal (Fig. 4.7). The highest total concentration of SCFA in faeces was with the 5% pectin group, which was also significantly higher than that of the 10% pectin group \( p<0.05 \).

4.3.5(c). Total caecal

Compared to the basal diet, total caecal SCFA was unaffected by the addition of fibre (Fig. 4.8). However, 5% pectin significantly increased the total caecal SCFA when compared to 10% bran \( p<0.05 \).

4.3.5(d). Total daily faecal excretion

The higher faecal weights with fibre supplemented diets (Fig. 4.5), resulted in an increase in the total faecal SCFA excreted per day (Fig. 4.9) compared to the basal diet. The high faecal concentration of SCFA with
Fig. 4.8. Total caecal SCFA from rats fed CRM(x), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or 6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B) were significantly different (t-test); P < 0.05.
Fig. 4.9. Total daily faecal excretion of SCFA from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or 6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (A, B, C) were significantly different (t-test); p < 0.05.
5% pectin resulted in a high daily excretion of SCFA with this diet.

4.3.5(e). Caecal molar proportions

Acetate was the principal SCFA in all groups. The molar proportion of acetate (Fig. 4.10) increased and the molar proportion of butyrate (Fig. 4.12) decreased proportionately in caecal material from rats fed 10% pectin. The molar proportions of SCFA were not influenced by the 10% bran or the pectin/bran mixed diets. The 5% pectin diet significantly increased the molar proportion of propionate (<p<0.05) (Fig. 4.11), but the molar proportions of acetate and butyrate were similar to those of the basal diet. CRM(X) yielded a high proportion of butyrate both pre-trial and after 4 weeks (Fig. 4.12).

4.3.6. Histology

There were no differences in the mucosal thickness for fibre supplemented diets compared to the basal diet (Table 4.2). Pectin and the pectin/bran mixed diets caused a significant decrease in the muscle thickness of the proximal colon by 44% and 39% respectively. The muscle thickness in the distal colon increased by 64% with the bran diet and by 70% with the pectin/bran mixed diet compared to the basal.
Fig. 4.10. Molar proportion of acetate \([\text{mmol SCFA (mol total SCFA)}^{-1}]\) in caecal material from rats fed CRM\(\text{x}\), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or +6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter \((^a-b)\) were significantly different (t-test); \(p<0.05\).
Fig. 4.11. Molar proportion of propionate [mmol SCFA (mol total SCFA)$^{-1}$] in caecal material from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or 6 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (a, b) were significantly different (t-test); p<0.05.
Fig. 4.12. Molar proportion of butyrate [mmol SCFA (mol total SCFA)\(^{-1}\)] in caecal material from rats fed CRM(X), basal, and basal supplemented with 10% bran, 10% pectin, 5% bran mixed with 5% pectin, and 5% pectin. Each bar represents mean ± SEM for 5 or 16 rats. Data were analysed with one-way analysis of variance and means not sharing the same letter (\(^a\), \(^b\)) were significantly different (t-test); p<0.05.
Table 4. Total muscle and mucosal thickness of proximal and distal colon from rats fed basal, and basal supplemented with 10% wheat bran, 10% pectin, and 5% pectin mixed with 5% wheat bran.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Proximal</th>
<th>Distal</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>214</td>
<td>379</td>
<td>293</td>
<td>131</td>
</tr>
<tr>
<td>(44)</td>
<td>(25)</td>
<td>(16)</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td>10% Bran</td>
<td>300</td>
<td>365</td>
<td>226</td>
<td>215 *</td>
</tr>
<tr>
<td>(52)</td>
<td>(12)</td>
<td>(41)</td>
<td>(20)</td>
<td></td>
</tr>
<tr>
<td>10% Pectin</td>
<td>230</td>
<td>338</td>
<td>164 *</td>
<td>153</td>
</tr>
<tr>
<td>(7)</td>
<td>(32)</td>
<td>(20)</td>
<td>(45)</td>
<td></td>
</tr>
<tr>
<td>5% Pectin/5% bran</td>
<td>259</td>
<td>371</td>
<td>178 ***</td>
<td>223 *</td>
</tr>
<tr>
<td>(27)</td>
<td>(6)</td>
<td>(11)</td>
<td>(27)</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as means (SEM in parentheses).
In order basal, bran, pectin, pectin/bran: n=; proximal mucosa: 3, 8, 5, 6; distal mucosa: 6, 9, 5, 4; proximal muscle: 3, 8, 5, 6; distal muscle: 5, 9, 5, 4. The mean of six observations was calculated for each section.
Significantly different from the basal (unpaired t-test); * p< 0.05, *** p< 0.001.
CHAPTER 5.

INFLUENCE OF FIBRE LEVEL AND HEAT-TREATED FIBRE ON

COLONIC METABOLISM AND FAECAL WEIGHT
5.1. Aim

The objective of this study was to investigate the effect of the fibre level and heat-treatment of CRM(X) on colonic metabolism and faecal weight.

5.2. Materials and methods

5.2.1. Animals and diets

CRM(X) diet (Section 2.1., Tables 2.4. and 2.5) is an expanded type of pellet diet. This diet was subjected to high temperatures, about 180°C and 85°C during the expansion and pelleting processes respectively. CRM(X) without heat treatment was supplied by Labsure, Croydon. Basal diet supplemented with 20% wheat bran was formulated to give a similar crude fibre level to CRM(X) (Tables 5.1 and 5.2).

Rats about 8 weeks old were divided into 3 groups of 6 and housed in pairs. Rats were fed one of 3 diets, heat treated (pelleted) CRM(X), raw (unpelleted) CRM(X), and basal supplemented with 20% wheat bran. Animals were fed for 28 days. Animal procedures are described in Section 2.1.

Procedures for sample collection and analysis of SCFA are described in Sections 2.3 and 2.5, respectively.
Table 5.1. Level of constituents in the basal diet supplemented with 200 g kg\(^{-1}\) wheat bran.

<table>
<thead>
<tr>
<th>Dietary constituent</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapro A</td>
<td>15.4</td>
</tr>
<tr>
<td>Soya oil</td>
<td>2.2</td>
</tr>
<tr>
<td>Cornflour</td>
<td>57.4</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20.0</td>
</tr>
<tr>
<td>Premix</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Formulation of diet carried out by Special Diets Services, Witham, Essex.

Table 5.2. Composition of basal diet alone and supplemented with 200 g kg\(^{-1}\) wheat bran.

<table>
<thead>
<tr>
<th>Dietary Constituent (%)</th>
<th>Basal diet</th>
<th>20% bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Analyses carried out by Special Diets Services, Witham, Essex.
5.3. RESULTS

5.3.1. Body weight gain

Feeding raw CRM(X) was associated with a significantly reduced body weight gain and hence, a reduced final body weight compared to heat treated CRM(X) and 20% bran (Table 5.3).

5.3.2. Weight of caecal contents

There was no significant difference between both CRM(X) diets for wet and dry weights of caecal contents in relation to body weight (Table 5.3), whereas, feeding 20% bran resulted in a significantly lower weight of caecal contents compared to the two CRM(X) diets (p<0.05).

5.3.3. Caecal-sac weight

Caecal-sac weights in relation to body weight (Table 5.3) were similar for rats fed 20% bran, heat-treated CRM(X) and raw CRM(X).

5.3.4. Dietary fibre intake and stool weight

The daily dietary fibre intake per rat was calculated from food intake (Table 5.4). The dietary fibre content of wheat bran was 38% (Section 2.1, Table 2.3). Faecal output in relation to daily dietary fibre intake was similar for all diets, except raw CRM(X).
Table 5.3. Wet and dry caecal contents, caecal tissue and body weight in adult rats fed raw and heat treated CRM(X), and basal diet supplemented with 20% wheat bran.

<table>
<thead>
<tr>
<th>Diet</th>
<th>20% bran (n=6)</th>
<th>Raw (n=6)</th>
<th>heat treated (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>106^a</td>
<td>94^a</td>
<td>107^a</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>2</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Wet caecal contents (g kg^-1) **</td>
<td>7.15^a</td>
<td>11.95^a</td>
<td>12.61^a</td>
</tr>
<tr>
<td></td>
<td>(0.16)</td>
<td>(0.95)</td>
<td>(0.40)</td>
</tr>
<tr>
<td>Dry caecal contents (g kg^-1) **</td>
<td>1.10^a</td>
<td>1.92^a</td>
<td>2.23^a</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.25)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>Caecal-sac (g kg^-1) **</td>
<td>1.00^a</td>
<td>0.90^a</td>
<td>0.98^a</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

Data are given as means (SEM in parentheses).

"** Expressed per kg body weight.

Means within the same horizontal row not sharing a common superscript differed significantly (Mann-Whitney U test; p < 0.05).
Table 5.4. Dietary fibre intake and dry faecal weight for rats fed raw and heat treated CRM(X), basal, and basal supplemented with either 10% or 20% wheat bran.

<table>
<thead>
<tr>
<th></th>
<th>+ Basal</th>
<th>10%</th>
<th>20%</th>
<th>Raw</th>
<th>Heat treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry food intake (g 3 days⁻¹)</td>
<td>86.0 (0.3)</td>
<td>83.0 (3.0)</td>
<td>84.0 (3.0)</td>
<td>54.0 (2.5)</td>
<td>68.0 (1.0)</td>
</tr>
<tr>
<td>Dry faecal weight (g 3 days⁻¹)</td>
<td>3.7 (0.4)</td>
<td>6.5 (0.2)</td>
<td>9.7 (0.3)</td>
<td>12.0 (0.5)</td>
<td>12.7 (0.6)</td>
</tr>
<tr>
<td>Dry faecal increment (g 3 days⁻¹)</td>
<td>3.2 (0.2)</td>
<td>6.7 (0.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Fibre intake</strong> (g 3 days⁻¹)</td>
<td>3.1 (0.1)</td>
<td>6.4 (0.2)</td>
<td>8.6 (0.4)</td>
<td>10.8 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Dry faecal increment fibre intake⁻¹</td>
<td>1.03a (0.08)</td>
<td>1.02a (0.03)</td>
<td>1.40a (0.07)</td>
<td>1.18a (0.03)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means of six observations (SEM in parentheses), n=5.

**Dietary fibre content of CRM(X) is 16%, and wheat bran is 38% (Section 2.1, Tables 2.3 and 2.4).** Data were analysed using one-way analysis of variance and means not sharing the same letter differed significantly (t-test); p < 0.05.
5.3.5. **Caecal short-chain fatty acids**

The concentration of propionate (Table 5.5) was increased in caecal contents from rats fed raw CRM(X) compared to those fed heat treated CRM(X) (p<0.05), the total SCFA concentrations were not significantly different. The total SCFA per caecum (Table 5.5) were similar for both CRM(X) diets, but reduced in the 20% wheat bran fed rats. The molar proportions of SCFA in caecal contents (Table 5.6) were similar for both CRM(X) diets. The molar proportion of propionate was significantly increased in the rats fed the 20% bran diet (p<0.05) compared to those fed the CRM(X) diets.
Table 5.5. Concentration and total SCFA in dry caecal material from adult rats fed raw and heat treated CRM(X), and basal supplemented with 20% wheat bran.

<table>
<thead>
<tr>
<th>Diet</th>
<th>20% bran (n=6)</th>
<th>Raw (n=6)</th>
<th>heat treated (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20% bran (n=6)</td>
<td>Raw (n=6)</td>
<td>heat treated (n=5)</td>
</tr>
<tr>
<td>Acetate (µmol g⁻¹)</td>
<td>251a (23)</td>
<td>350a (22)</td>
<td>317ab (16)</td>
</tr>
<tr>
<td>Propionate (µmol g⁻¹)</td>
<td>96a (4)</td>
<td>93a (9)</td>
<td>68a (4)</td>
</tr>
<tr>
<td>Butyrate (µmol g⁻¹)</td>
<td>86a (4)</td>
<td>125ab (20)</td>
<td>138ab (11)</td>
</tr>
<tr>
<td>Total SCFA (µmol g⁻¹)</td>
<td>457a (25)</td>
<td>586a (44)</td>
<td>537ab (15)</td>
</tr>
<tr>
<td>Total caecal SCFA (µmol caecum⁻¹)</td>
<td>184a (14)</td>
<td>408a (55)</td>
<td>487a (15)</td>
</tr>
</tbody>
</table>

Data are shown as means (SEM in parentheses). Means within the same horizontal row not sharing a common superscript differed significantly (Mann-Whitney U test; p < 0.05).
Table 5.6. Molar proportions [mmol SCFA (mol total SCFA⁻¹)] of SCFA in dry caecal material from adult rats fed raw and heat treated CRM(X), and basal supplemented with 20% wheat bran.

<table>
<thead>
<tr>
<th>Diet</th>
<th>20% bran (n=6)</th>
<th>not heated (n=6)</th>
<th>heated (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>544a</td>
<td>601b</td>
<td>596ab</td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(17)</td>
<td>(13)</td>
</tr>
<tr>
<td>Propionate</td>
<td>212a</td>
<td>160b</td>
<td>130b</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(14)</td>
<td>(7)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>189a</td>
<td>201ab</td>
<td>248b</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(20)</td>
<td>(17)</td>
</tr>
</tbody>
</table>

Data are shown as means (SEM in parentheses).
Means within the same horizontal row not sharing a common superscript differed significantly (Mann-Whitney U test; p < 0.05).
CHAPTER 6.

COMPARISON BETWEEN RATS WEANED ONTO TEST DIETS AND RATS CHANGED OVER TO TEST DIETS AS ADULTS.
6.1. **Aim**

The aim of this study was to compare rats which had been weaned directly onto the test diets, with rats which were raised on the laboratory stock diet prior to feeding the test diets.

6.2. **Materials and methods**

Male rats, aged 6 weeks old and which had been maintained on CRM(X) prior to experimentation, are described in Chapter 4. For the weaning experiment, 3 groups of both mothers and litters were fed 1 of 3 test diets from day 12 after parturition to avoid the young prematurely eating the mother's original diet. At weaning (21 days old), the male young were selected, allocated to groups of 5 and continued on the same diet for 7 weeks. Therefore, the weaned rats and the adult rats were 10 weeks old at the end of the experiment. The test diets were basal, and basal supplemented with 10% wheat bran or 10% pectin. Animal procedures and diets are described in Section 2.1.

Procedures for sample collection and analysis of SCFA are described in Sections 2.3 and 2.5 respectively.
6.3. RESULTS

In this section, the adult groups are discussed only in comparison to the corresponding weanling groups. Results for adults are considered in more detail in Section 4.3.

6.3.1. Body weight gain

Weanlings fed 10% pectin showed a decreased growth rate (Fig. 6.1) compared to both the basal and 10% bran diets. As a result, final body weight was significantly reduced (<p<0.05) (Table 6.1).

6.3.2. Weight of caecal contents

The wet and dry weight of caecal contents in relation to final body weight (Table 6.1), were significantly increased with 10% pectin for both weanlings and adults. Any one diet had the same effect on the wet and dry weights of caecal contents, in relation to body weight, for both weanlings and adults.

6.3.3. Caecal-sac weight

Within the weanling groups, 10% pectin increased the caecal-sac weight (<p<0.05) (Table 6.1), and this was significantly greater when compared to the adult group fed 10% pectin (<p<0.001). Adults fed the basal diet showed a significantly increased caecal-sac weight compared to animals weaned directly onto this diet.
Fig. 6.1. Growth curve for weanling rats fed basal, and basal supplemented with 10% of either wheat bran or pectin.
Table 6.1. Wet and dry caecal contents, caecal tissue and body weight in weanling and adult rats fed basal, and basal supplemented with 10% wheat bran or 10% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weanlings 1</th>
<th>Adults 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Bran*</td>
</tr>
<tr>
<td>Final body wt. (g)</td>
<td>288alent</td>
<td>329B</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Wet caecal contents (g) **</td>
<td>8.62A</td>
<td>10.49A</td>
</tr>
<tr>
<td></td>
<td>(0.91)</td>
<td>(0.45)</td>
</tr>
<tr>
<td>Dry Caecal contents (g)**</td>
<td>1.58A</td>
<td>1.91A</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Caecal-sac (g)**</td>
<td>1.33A</td>
<td>1.44A</td>
</tr>
<tr>
<td></td>
<td>(0.10)</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

Data are given as means of five rats (SEM in parentheses) or * for six rats.
Rats on experimental diet from weaning.
Rats on stock diet [CRM(X)] from weaning and transferred at 6 weeks old to experimental diet.
** expressed per kg body weight.
One-way analysis of variance was carried out separately on weanlings and on adults and means within the same horizontal row not sharing a common superscript differed significantly (t-test): p < 0.05 (a,b,c for weanlings and d,e,f for adults).
Adult values marked with asterisks were significantly different from the corresponding weanling value (unpaired t-test): ** p < 0.01, *** p < 0.001.
The caecal-sac weights were similar for both adults and weanlings fed 10% bran.

6.3.4. Caecal short-chain fatty acids

6.3.4(a). Concentration

When rats were weaned directly onto the basal diet, the acetate and propionate concentrations were significantly decreased (both p<0.01) compared to the adults fed basal diet (Table 6.2). Therefore, the total SCFA concentration for weanlings fed basal was reduced compared to the adults (p<0.01). Conversely, weanlings fed 10% pectin showed increased concentrations of propionate (p<0.001) and butyrate (p<0.01) compared to adults fed 10% pectin, and the total SCFA concentration increased for weanlings fed 10% pectin compared to the corresponding adult group (p<0.05). Total SCFA concentrations were similar for both weanlings and adults fed 10% bran, although the butyrate concentration was significantly increased (p<0.01) for the weanlings.

6.3.4(b). Total caecal SCFA

Within the weanling groups 10% pectin significantly increased the total SCFA per caecum compared to the basal diet (p<0.05) but not when compared to 10% bran (Table 6.2). Total SCFA per caecum were similar for both weanlings and adults fed the 10% bran and 10% pectin diets.
Table 6. Concentration and total SCFA in dry caecal material from weanling and adult rats fed basal, and basal supplemented with 10% wheat bran or 10% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weanlings</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Bran +</td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol g⁻¹)</td>
<td>284</td>
<td>297</td>
</tr>
<tr>
<td></td>
<td>(26)</td>
<td>(6)</td>
</tr>
<tr>
<td>Propionate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol g⁻¹)</td>
<td>82</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(9)</td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol g⁻¹)</td>
<td>103</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(4)</td>
</tr>
<tr>
<td>Total SCFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol g⁻¹)</td>
<td>474</td>
<td>520</td>
</tr>
<tr>
<td></td>
<td>(41)</td>
<td>(12)</td>
</tr>
<tr>
<td>Total caecal SCFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol caecum⁻¹)</td>
<td>220</td>
<td>330E**</td>
</tr>
<tr>
<td></td>
<td>(37)</td>
<td>(43)</td>
</tr>
</tbody>
</table>

Data are given as means of five rats (SEM in parentheses) or * for six rats.

1 Rats on experimental diet from weaning.

2 Rats on stock diet [CRM(X)] from weaning and transferred at 6 weeks old to experimental diet.

One-way analysis of variance was carried out separately on weanlings and on adults and means within the same horizontal row not sharing a common superscript differed significantly (t-test); * p < 0.05 (A, B, C for weanlings and D, E, F for adults).

Adult values marked with asterisks were significantly different from the corresponding weanling value (unpaired t-test); * p < 0.05, ** p < 0.01, *** p < 0.001.

-76-
6.3.4(c). **Molar proportions**

Caecal molar proportions are shown in Table 6.3. Within the weanling groups the molar proportion of propionate increased significantly with 10% pectin compared to the basal diet ($p<0.05$), and was significantly greater than the corresponding adult group ($p<0.001$). Also, the acetate molar proportion decreased ($p<0.01$) and the butyrate molar proportion increased ($p<0.05$) in weanlings fed 10% pectin compared to adults fed the same diet. In weanlings, the 10% bran diet increased the molar proportion of butyrate ($p<0.001$) and decreased the proportion of acetate ($p<0.05$) compared to adults fed 10% bran. Despite the decreased total SCFA concentration in caecal contents in weanlings fed basal compared to the adults (Table 6.2), the molar proportions of SCFA were similar (Table 6.3).
Table 6.3. Molar proportions [mmol SCFA (mol total SCFA)\(^{-1}\)] of SCFA in dry caecal material from weanling and adult rats fed basal, and basal supplemented with 10% wheat bran or 10% pectin.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weanlings ¹</th>
<th>Adults ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Bran *</td>
</tr>
<tr>
<td>Acetate</td>
<td>600(^a)</td>
<td>571(^a)</td>
</tr>
<tr>
<td></td>
<td>(23)</td>
<td>(6)</td>
</tr>
<tr>
<td>Propionate</td>
<td>176(^a)</td>
<td>159(^a)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(14)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>214(^ab)</td>
<td>263(^b)</td>
</tr>
<tr>
<td></td>
<td>(27)</td>
<td>(11)</td>
</tr>
</tbody>
</table>

Data are given as means of five rats (SEM in parentheses) or ¹ for six rats.

¹ Rats on experimental diet from weaning.

² Rats on stock diet [CRM(X)] from weaning and transferred at 6 weeks old to experimental diet.

One-way analysis of variance was carried out separately on weanlings and on adults and means within the same horizontal row not sharing a common superscript differed significantly (t-test); \(p < 0.05\) (\(^a\),\(^b\),\(^c\) for weanlings and \(^o\),\(^e\),\(^f\) for adults).

Adult values marked with asterisks were significantly different from the corresponding weanling value (unpaired t-test); \(^* p < 0.05\), \(^** p < 0.01\), \(^*** p < 0.001\).
CHAPTER 7.

INFLUENCE OF WHEAT BRAN AND PECTIN ON CAECAL AND FAECAL
TOTAL WATER CONTENT AND BOUND WATER.
7.1. Aim

This study investigated the manner in which water was bound to the caecal contents and faeces from rats fed wheat bran or pectin, using an in vitro method. This gave further insight into the mechanisms of faecal bulking.

7.2. MATERIALS AND METHODS

7.2.1. Animals and diets

Male rats, aged 6 weeks old, were divided into 3 groups of 6, housed in pairs, and fed basal diet, or basal supplemented with 10% of either wheat bran or pectin for 28 days. Animal procedures and diets are described in Chapter 2, Section 2.1.

7.2.2. Collection of caecal and faecal material

Collection of faecal and caecal material is described in Chapter 2, Section 2.3. In addition, freshly voided faeces were collected, weighed, frozen and stored at -20°C.

7.2.3. Water content of faecal and caecal material

Fresh caecal and faecal material were weighed, frozen at -20°C, freeze-dried and re-weighed to determine the water content.
7.2.4. **Suction method**

Dialysis tubing (Medicell International Ltd, London) size 4-22/32" was cut into 100mm lengths and soaked overnight in a solution of sodium azide (1g dm\(^{-3}\)) and one end of the bag was tied with waxed dental floss. Fresh faecal and caecal material were hydrated overnight in a solution of sodium azide and aliquots (equivalent to 100mg dry weight) placed into the dialysis tubing followed by 1cm\(^3\) sodium azide solution. The other end of the dialysis tubing was tied off before placing the bag into a 150cm\(^3\) conical flask. 50cm\(^3\) of PEG 10 000 (BDH chemicals, Glasgow), of known osmolality (Section 7.2.7), were added to each flask. A dialysis bag with 1cm\(^3\) sodium azide solution was used as a control. Flasks were sealed and shaken in an orbital shaker at room temperature. After 72 hours the bags were removed, blotted, placed in a dry sealed universal vial and weighed. The wet weight of the sample was determined by subtracting the weight of both the wet bag and vial. The bag was cut open placed in a weighed universal, frozen at -20°C then freeze-dried. The dry weight of sample was determined by subtracting the weight of both the vial and freeze-dried dialysis bag. Each determination was carried out in duplicate. The WHC (or bound water) were expressed as g water held per g dry weight sample.
7.2.5. Choice of osmoticum

An osmoticum is a compound used to generate an osmotic potential across the dialysis membrane. An osmoticum must be:

(a) Water soluble

(b) Impermeable to the dialysis membrane

Ideally a compound should be of molecular weight greater than 10 000. In previous experiments PEG 6000 was used, the highest molecular weight available at that time (Robertson & Eastwood, 1981b) but at this molecular weight penetration of the membrane was estimated to be less than 5% after 72 hours equilibrium. In this present experiment, PEG 10 000 (BDH Chemicals, Glasgow) and smaller sample sizes are used (100mg rather than 500mg equivalent dry weight). McBurney et al. (1985) used PEG 3350, however their dialysis bags had a molecular weight cut-off point of 2000.

7.2.6. Choice of suction pressures

An osmotic difference of approximately 2atm. (89 mosmol dm\(^{-3}\)) exists across the colonic mucosa (McBurney et al., 1985), therefore this was used as the physiological suction pressure. Increasing the suction pressure to 4atm. (178 mosmol dm\(^{-3}\)) would give further indication as to how strongly water was held by the caecal and faecal material.
7.2.7. Measurement of osmotic potential development

Increasing concentrations of PEG 3350, 6000, 10 000 and 20 000 resulted in a curvilinear increase in osmolality, measured by freeze-point depression using an osmometer (Fig. 7.1). Suction pressures of 2 atm. (89 mosmol dm$^{-3}$) and 4 atm. (178 mosmol dm$^{-3}$) were produced by adding 110g and 148g, of PEG 10 000 per litre sodium azide solution respectively. The osmolality of sodium azide (30 mosmol dm$^{-3}$) was taken into account when calculating the suction pressures.

7.2.8. Statistical treatment

Results were analysed using the unpaired t-test and means were considered different at p<0.05, P<0.01 and p<0.001 levels.
Fig. 7.1. Osmolality of polyethylene glycol of different molecular weights at different concentrations.
7.3. RESULTS

7.3.1. WHC of wheat bran and pectin

WHC of wheat bran and pectin are shown in Table 7.1. At 2 atm. pectin held about 4 times as much water as wheat bran. The WHC of pectin was further reduced at 4 atm. (P<0.001) compared to the 2 atm. value for pectin. However, wheat bran had similar WHC at both pressures. The more strongly the water is held by the fibre the greater the gradient of water loss. Therefore, pectin had a stronger association with water compared to wheat bran.

7.3.2. Water content and bound water of caecal material

The water contents of caecal material on all diets (Table 7.2.) were greater than the bound water measured by the suction method. The caecal water content of pectin-fed rats was higher compared to the basal group (P<0.01). Although the bound water measured in vitro increased with the fibre-supplemented diets the differences were not significant. Therefore, each diet had a similar bound water held per g caecal contents measured by the suction technique.

7.3.3. Water content and bound water of faecal material

Results are shown in Table 7.3. None of the diets caused diarrhoea but faecal pellets from rats fed pectin appeared wetter and elongated compared to those produced
Table 7.1. Water-holding capacity (WHC) of wheat bran and pectin measured by the suction method

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>4.3</td>
<td>0.7</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Pectin</td>
<td>16.4</td>
<td>0.2</td>
<td>10.7 ***</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are means of triplicate observations. Significantly different from 2 atm, value *** $p < 0.001$ (unpaired t-test).
Table 7.2. Water content and bound water of fresh caecal material from rats fed basal, or basal supplemented with either 10% wheat bran or pectin.

<table>
<thead>
<tr>
<th>Suction Method</th>
<th>Bound water (g water g dry contents⁻¹)</th>
<th>Water Content of Fresh Caecal Material (g water g dry contents⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suction Pressure</td>
<td>(mosmol l⁻¹)...89</td>
<td>(atm.)...2</td>
</tr>
<tr>
<td>Diet</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Basal</td>
<td>1.16 0.19</td>
<td></td>
</tr>
<tr>
<td>10% Pectin</td>
<td>1.41 0.24</td>
<td></td>
</tr>
<tr>
<td>10% Bran</td>
<td>1.64 0.22</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as means of 6 observations (each observation was duplicated for the suction method only), n=5

Significantly different from the basal ** p < 0.01 (unpaired t-test),
Table 7.3. Water content and bound water of fresh faecal material from rats fed basal, or basal supplemented with either 10% wheat bran or 10% pectin.

| Suction Pressure | Bound water (g water g dry contents⁻¹) | Suction Method | Water Content of Fresh Faeces (g water g dry contents⁻¹) | % Water |
|------------------|------------------------------------------|----------------|------------------------------------------------------|
| (mosmol 1⁻¹)     |                                          | Mean SEM      | Mean SEM    | Mean SEM | Mean SEM |
| 89               |                                           | 1.22 0.07     | 0.84 0.06   | 1.55 0.09 | 60.6 1.3 |
| (atm)            |                                          | 1.49* 0.08    | ** 0.94 0.10 | ** 2.38 *** 0.08 | 70.2 0.6 |
| 2                |                                          | 1.12 0.08     | 1.25* 0.12  | 1.45 0.10 | 58.8 1.9 |

Data are means of 6 observations (each observation was duplicated for the suction method only). Significantly different from the basal *p < 0.05, **p < 0.01, ***p < 0.001; (unpaired t-test).

* n=5
** n=9
with a bran diet. 10% pectin increased the water content of fresh faeces \( (p<0.001) \), and bound water \( (2\text{atm.}) \). Wheat bran had a similar water content and bound water at 2 atm. to the basal diet.

7.3.4. **Influence of diet on total water content and bound water in the caecum**

Pectin significantly increased the wet and dry weights of caecal contents \( (p<0.05) \) and total water per caecum \( (P<0.05) \) (Table 7.4). However, the total bound water increased with 10% bran \( (p<0.01) \) and 10% pectin \( (p<0.01) \) compared to the basal diet. By difference, there was more free water available for absorption at 2 atm. from the caecum of pectin-fed rats compared to the basal.

7.3.5. **Influence of diet on total water content and bound water excreted in faeces**

Fibre supplemented diets significantly increased daily wet stool weight and total water excreted per day (Table 7.5). However, only wheat bran significantly increased the dry stool weight \( (P<0.05) \). Total bound water excreted per day was significantly increased with both fibre diets. By difference there was more free water available for absorption at 2atm. from faeces of pectin-fed rats compared to the basal.
### Table 7.4: Influence of wheat bran and pectin on total water content and bound water in the caecum

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>10% bran</th>
<th>10% pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>wet weight (g)</td>
<td>2.9</td>
<td>3.0</td>
<td>5.5 **</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>dry weight (g)</td>
<td>0.6</td>
<td>0.7</td>
<td>1.0 *</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>total water (g caecum⁻¹)</td>
<td>2.3</td>
<td>2.4</td>
<td>4.6 *</td>
</tr>
<tr>
<td></td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>* bound water (g g dry weight⁻¹)</td>
<td>1.2</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>* bound water (g caecum⁻¹)</td>
<td>0.7</td>
<td>1.2 **</td>
<td>1.4 **</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

Data are shown as means (SEM in parentheses)
* after 72 hours at 2 atm, pressure.
Significantly different from basal * p < 0.05, ** p < 0.01 (unpaired t-test).
Table 7.5. Influence of wheat bran and pectin on total water content and bound water excreted in faecal material

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>10% bran</th>
<th>10% pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>wet weight (g day⁻¹)</td>
<td>3.3</td>
<td>5.4 **</td>
<td>5.2 **</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.1)</td>
<td>(0.3)</td>
</tr>
<tr>
<td>dry weight (g day⁻¹)</td>
<td>1.3</td>
<td>2.2 *</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>total water (g day⁻¹)</td>
<td>2.0</td>
<td>3.3 **</td>
<td>3.6 ***</td>
</tr>
<tr>
<td></td>
<td>(0.2)</td>
<td>(0.1)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>+ bound water</td>
<td>1.2</td>
<td>1.1</td>
<td>1.5 *</td>
</tr>
<tr>
<td>(g g dry weight⁻¹)</td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>+ bound water</td>
<td>1.5</td>
<td>2.4 **</td>
<td>2.3 **</td>
</tr>
<tr>
<td>(g day⁻¹)</td>
<td>(0.2)</td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
</tbody>
</table>

Data are shown as means (SEM in parentheses),
* after 72 hours at 2 atm. pressure,
Significantly different from basal * p < 0.05, ** p < 0.01, *** p < 0.001 (unpaired t-test),
CHAPTER 8.

GENERAL DISCUSSION
The physical and chemical properties of fibre, and the extent to which it is fermented, are important in determining the physiological effects of dietary fibre in the large bowel (Cummings, 1982). The influence on stool weight differs with the type of fibre, and depends on chemical structure, solubility, particle size, and lignification. The physical effects of dietary fibre in the colon have been best demonstrated with studies using coarse wheat bran (Smith, 1982). For contrast, pectin was chosen in this present investigation, since it is known to be relatively more susceptible to fermentation (Cummings et al., 1979). Therefore, pectin may influence colonic metabolism and bowel function by the end-products of fermentation.

Any factor which may alter the structure of fibre, such as heat treatment, may be expected to alter the physiological effect of fibre. In addition, there are few reports in the literature about the influence of other fibre components in the diet, fibre level, previous diet, and adaptation, on the fermentation of dietary fibre.

To date, most of the studies on the metabolism and faecal bulking properties of dietary fibre have been in humans. In health, the human caecum is inaccessible to study fibre metabolism, and so investigations have been somewhat indirect by sampling faeces, blood, or breath hydrogen and methane (Mckay et al., 1981). Furthermore,
there is considerable variation between individuals. Previous studies in this laboratory have demonstrated that the rat is a useful model for studying the effect of dietary fibre on caecal metabolism and faecal output (McLean Ross et al., 1984; Walter et al., 1986). The model is the adult male rat which has been maintained on a laboratory stock diet prior to experimentation. The experimental procedures involve supplementing a fibre-free basal diet with 10% fibre. A period of 4 weeks is suggested to be sufficient for the investigation of novel dietary fibres (Walter et al., 1986). This model was used in the present studies, but was further developed (see later) to eliminate the use of non-standardised stock diets, by weaning animals directly onto the test diets.

Information on the colonic metabolism of dietary fibre was assessed by measuring the weights of the caecal-sac and contents, bacterial biomass (DAPA), bacterial numbers, and the concentration and relative proportions of SCFA. Similar measurements were determined in the faeces. Also, the manner in which water was held by caecal material and faeces was determined.

8.1. Effects of wheat bran and pectin in the adult rat

8.1.1. Influence on feed intake and growth

Fibre was added to the complete basal diet such that the ratio of essential nutrients to energy was not
changed, and animals were able to satisfy their energy intake, if necessary, by increasing food intake (Bright-See et al., 1978). This procedure is based on the assumption that fibre provides little or no energy to the animal. This may be more accurate for relatively inert fibres, such as cellulose, but energy may be obtained from fermentable fibres by means of absorbed SCFA. It has been estimated that 30g of carbohydrate from the diet and intestinal secretions would yield 300mmol of SCFA each day in the human colon (Cummings & Branch, 1986). However, Rowland et al. (1986) point out that energy may be lost in the faeces by increased bacterial biomass and increased bacterial use of nutrients. Therefore, the authors recommend that the fibre is added to the complete basal diet until this balance in energy has been determined.

Animals consumed less of the pectin supplemented diets and also had reduced weight gains compared to the basal diet. Gelling polysaccharides, such as guar gum, can result in a decrease in food intake especially at 20% and 40% additions in the rat (Elsenhans et al., 1981). The addition of 10% gum arabic to the same basal diet as used in the present study, did not affect body weight gain (Walter et al., 1986). In comparable studies, reduced weight gain in pectin-fed animals is well documented. This has led to the conclusion that the SCFA produced from the breakdown of pectin do not contribute significant amounts of energy to young rats.
(Hove & King, 1979). Judd and Truswell (1985) have reported that weight gain is less marked with high molecular weight compared to low molecular weight pectin, and that molecular weight and hence viscosity are factors affecting food intake and weight gain. Pectins range from 30,000 to 300,000 molecular weight and in the present study, the pectin used was of an average molecular weight (determined by an inherent viscosity figure (Section 2, Table 2.2)). Reduced weight gain has also been reported with 10% high-methoxyl pectin compared to a stock diet (Illman et al., 1982). However, there are other reports of no adverse affect on weight gain with 5% high-methoxyl pectin (Rowland & Mallett, 1983; Thomsen et al., 1984). The reduced weight gain observed in this present study occurred mainly during the first 2 weeks of the experiment and was minimised with a longer period of feeding. Similarly, Scheibel et al. (1985) suggested that adaptation to 7.5% high-methoxyl pectin, assessed by food intake and growth measurements, occurred after 3 weeks, but since diets were isocaloric i.e. substituting starch with pectin, their results are not strictly comparable with this present study.

The reason for the reduction in caloric intake with pectin-supplemented diets is not clear. It does not appear to be due to a reduction in protein digestibility, since increasing the dietary casein levels did not influence the effect of pectin on feed
intake in the rat (Hove & King, 1979; Delorme & Gordon, 1984). In the present study, pectin-fed rats had full stomachs at post mortem which has also been observed by Judd and Truswell (1985). They suggest that inhibition of food intake may be by a bulk limiting effect due to stomach distention and the delayed emptying of gastric contents, as seen with guar gum (Leeds et al., 1979).

Ideally, rats should be fed levels of fibre which can be tolerated by humans, however, high levels were used in the present study in order to identify a clear response. Additions of 10% wheat bran can be tolerated in human diets (Spiller et al., 1986), but pectin is only tolerated at a lower level. For instance, stomach discomfort has been reported in humans after a daily ingestion of 15g (about 3%) of pectin added to a normal diet (Vargo et al., 1985).

8.1.2. Influence on caecal metabolism.

The consumption of pectin-supplemented diets was associated with an increase in the weight of wet caecal contents. The effect was dose-related with 10% pectin producing a greater increase than 5% pectin. There was a 2-fold increase in the dry weight of the caecal contents in rats fed 10% pectin. This increase was considerably less than the 4-fold increase observed with 10% gum arabic, supplemented into the same basal diet as the present study, and fed for 4 weeks (Walter et al., 1986). The degree of caecal enlargement will also
depend on the type of basal diet, since the addition of gum arabic to an elemental diet caused a 4-fold increase in wet weight of caecal contents whereas, there was only a slight increase when it was added to a stock diet (Mclean Ross et al., 1984). A recent study has also demonstrated a greater increase in the weight of caecal contents when guar gum was added to a basal diet containing albumin rather than casein (Southon et al., 1987). In the present study, wheat bran did not alter the weight of caecal contents which is in agreement with Walter et al. (1986). However, the pectin/bran mixed diet gave an intermediate weight for the wet caecal contents compared to pectin and wheat bran fed separately. It has been suggested that caecal enlargement is related to the osmotic content and not the bulk of the luminal contents (Leegwater et al., 1974).

Caecal enlargement usually occurs by three weeks after starting to feed the caecal enlarging supplement (Walker, 1978) and reverts to a normal size within four weeks after the animals are returned to a control diet (Leegwater et al., 1974). Therefore, maximal caecal enlargement should have been achieved in this current study. The caecal weight adaptation to 15% levels of two closely related substrates, gum arabic and guar gum, appears to be more rapid with gum arabic and is achieved in 10 days (Tulung et al., 1987). Therefore, animals may adapt to the increase in fermentable fibre by
increasing caecal size in order to increase absorption and utilise energy from such diets.

In the gut, bacteria are closely associated with the digesta and it is therefore difficult to distinguish them from background debris with direct microscopic counts. Most methods for measuring bacterial biomass in natural heterogeneous habitats are subject to error (Clarke & B auchop, 1977) but measurements of DAPA, can be useful. However, if the DAPA method is used in isolation of other methods for measuring bacteria, then the results are difficult to interpret since DAPA is not present in all bacteria (Rhuland, 1960), varies in content depending on the species (Synge, 1953; Purser & Beuchler, 1966) and in certain circumstances bacteria may be excreted into the surrounding medium (Nikolic & Jovanovic, 1973) and may be metabolised by other colonic bacteria (Masson & Ling, 1986). Therefore, in this current study, in addition to measuring DAPA, the number of bacteria was determined using a plate-count technique. Valid measurements of bacterial numbers can be made with the plate-count method since the efficiency of recovery of bacteria has been reported to be about 95% (Veilleux & Rowland, 1981) which compared well with strict anaerobic roll-tubes (Hungate, 1969) and with direct microscopic counts of bacteria (Holdeman & Moore, 1975).

The present study observed a positive correlation between DAPA measurements and bacterial numbers ($r=0.45$, $p$
p<0.05). Hence, it could be assumed that the increased bacterial DAPA in the caecal contents of rats fed both 10% pectin or pectin/bran mixed diets reflected an increase in bacterial numbers. The increased caecal DAPA reflected the greater amount of available substrate for the growth of caecal bacteria. Rowland & Mallett (1983) have reported a 50% increase in the number of bacteria per caecum with 5% pectin and a 90% decrease with 5% carageenan. The increase in caecal DAPA with 10% gum arabic (Walters et al., 1986) was greater than that determined with 10% pectin in the present study. This difference was related to the greater increase in the weight of caecal contents with gum arabic compared to that with pectin.

The composition of the caecal flora of the Wistar rat was not investigated in this study. The intestinal microbial population of the rat may be comparable to that of humans (Donaldson, 1968). However, Veilleux and Rowland (1981) determined the faecal composition of bacteria in the Wistar rat and the Sprague Dawley rat. The community structure was different between the two rat strains for anaerobes, lactobacilli and streptococci, but the species composition of the communities were similar. The faecal bacterial population of the Wistar rat was composed of 51% anaerobes, 26% lactobacilli, and 22% streptococci. In general, the lactobacilli and streptococci are not such a significant proportion of the human faecal flora as in
the Wistar rat. Since DAPA is not present in lactobacilli or cocci then DAPA may only be present in about half of the colonic bacterial groups in the rat. Despite this, DAPA is a good indicator of bacterial biomass but it cannot be extrapolated to an absolute weight value.

The anaerobic:aerobic ratio decreased when the basal diet was supplemented with 10% wheat bran or 10% pectin, indicating that the bacterial populations were changed by these fibres. Vargo et al. (1985) also found a decrease in the anaerobe:aerobe ratio in human faeces after supplementing the diet with 15g of pectin for 21 days. However, most studies have failed to detect differences in the dominant types of human faecal flora in response to a change in diet and as a result, the colonic flora is generally considered to be stable (Moore et al., 1978; Savage, 1983). This may be explained by inadequate anaerobic methodology and storage of samples, and by the considerable variation between the flora of individuals which appears to be greater than any change that may be induced by diet. However, studies with animals have been more successful in detecting dietary induced changes in the intestinal bacterial composition (Drasar & Barrow, 1985).

The increase in the number of caecal bacteria was not paralleled by an increase in SCFA, since the 10% pectin diet and the basal diet produced similar amounts of caecal SCFA despite the basal diet having a lower
caecal bacterial biomass. Since the basal diet was a fibre-free diet then such a high concentration of SCFA might not have been expected. The basal diet, however, contained about 70% maize flour the major proportion being starch. It has been estimated that up to 20% of the starch in a human diet (Stephen et al., 1983) and 40% potato starch in a rat diet (Fleming & Vose, 1979) escapes digestion by the pancreatic amylase in the small intestine, and starch can be readily fermented by the colonic microflora (Macfarlane & Englyst, 1986). It is therefore possible that a considerable proportion of starch, in the basal diet used in this present study, reached the caecum for bacterial fermentation to SCFA. In addition, the fermentation of mucopolysaccharides, intestinal secretions and sloughed cells (Salyers & Leedle, 1983) may have also contributed to the caecal SCFA content. However, all these polysaccharides would also be available to the rats fed the fibre-supplemented diets as well as the extra available polysaccharides from the dietary fibre, and yet the caecal concentration of SCFA decreased with the 10% addition of pectin despite the increased bacterial biomass. It is not clear from the results if this was due to a difference in production, utilisation or absorption of SCFA. It may be that energy was used for bacterial growth rather than for the production of SCFA.

There are few fibre dose-response studies reported in the literature, and the influence of fibre-level on
the extent of fermentation appears to be controversial (Chapter 1, Section 1.6.3)). Hove and King (1979) showed a linear increase \((r=0.68)\) in total caecal SCFA in rats fed increasing levels, up to 10%, of low-methoxyl pectin for 4 weeks. Whereas, in the present study, the mass of caecal SCFA did not increase when high-methoxyl pectin was increased from 5 to 10%. If it is assumed that SCFA reflect the apparent digestibility of dietary fibre, then this would suggest that increasing the level of high-methoxyl pectin did not increase the fermentability.

The experiments of Hove and King (1979) differ from the present study in, the species of rat, type of basal diet, and degree of pectin methoxylation, and furthermore the rats were weanlings at the start of the experiment. Gilmore (1966) found that low-methoxyl pectin was less readily fermented than a high-methoxyl type. In contrast, Nyman and Asp (1982) reported an increase in the excretion of uronic acid from 19% to 25% in the faeces of Sprague Dawley rats fed low- and high-methoxyl pectin respectively. However, the increase was not significant due to a high variation between different rats. In addition, the level of low-methoxyl pectin did not significantly affect the faecal excretion of uronic acids when rats were fed diets containing 42 compared to 84g kg\(^{-1}\) low-methoxyl pectin (Nyman & Asp, 1985).
Although the caecum is the major site in the rat for the breakdown of fibre, fermentation also occurs in the colon. The amount of SCFA in the colonic digesta was not measured in the present study, but in general, the concentrations are less than in the caecum (Remy & Demigne, 1976; Mclean Ross et al., 1984). It may be that in the present study, the higher level of pectin caused an increase in the retention time so allowing a longer time for the fermentation of pectin. The study with 10% gum arabic produced about a 3-fold increase in total caecal SCFA compared to the basal diet (Walter et al., 1986) whereas, in the present study, 10% pectin gave a similar caecal SCFA to the basal diet. Furthermore, 10% pectin increased the dry faecal output by 40% (in relation to food intake) and 10% gum arabic had no influence on dry faecal weight. This would suggest that pectin was not fermented to the same extent as gum arabic.

In addition to starch, other non-fibrous components in the diet such as fat, sugars and protein may become trapped in the pectin gel in the upper intestine and be carried down to the caecum. This demonstrates the difficulty in determining the exact amount and type of substrates in the caecum apart from dietary fibre.

The total caecal SCFA in rats fed wheat bran was reduced compared to that of the 5% pectin-fed rats, but not significantly compared to the 10% pectin-fed rats. Cheng et al., (1987) have indicated that the aleurone
layer of wheat bran is more readily fermented compared to the highly lignified pericarp layer. Also, the starch in the wheat bran may also reach the colon for fermentation.

The pectin/bran mixture produced a higher concentration of SCFA compared to the single additions of 10% wheat bran and 10% pectin. Topping et al. (1985) also observed a greater concentration of caecal SCFA in rats fed 7% cellulose/7% gum arabic mixed diet compared to these fibres fed at single levels of 14%. The concentrations of SCFA for 14% gum arabic and 14% cellulose were similar, but the former diet gave a greater increase in the weight of caecal contents and hence a greater mass of total caecal SCFA. The authors suggested that since the total caecal SCFA for the cellulose/gum arabic mixed diet was intermediate between the cellulose and gum arabic fed separately then the mixed diet did not modify total fermentability. In this present study, the pectin/bran mixed diet gave similar caecal SCFA to both the 10% pectin and 10% bran diets suggesting that the combination of fibres increased the fermentability of one or both of the fibres. Demigne and Remesy (1985) were able to achieve high caecal SCFA in the rat by feeding diets containing a mixture of fibre types in moderate proportions.

In accordance with the present study, Rowland et al. (1986), have reported no difference in the caecal concentration of SCFA in rats fed 5% high-methoxyl
pectin compared to the basal diet. However, Thomsen et al. (1984) found a slight increase with weanling Sprague Dawley rats fed high-methoxyl pectin for 27 weeks compared to the basal diet.

Rats are nocturnal feeders and it was assumed that they consumed their food about the same time each day. All samples in these studies were taken at 10 a.m. and SCFA may not have been at maximal concentration, since the amount increases in the caecum from zero to 2 hours after the last meal, after which it decreases (Yang et al., 1970). Animals were fed ad libitum and so a variation in SCFA concentrations might be expected. However, the variation was greatest in particular, with those rats which were fed the basal diet and the 10% pectin diet. This may be due to the animals being at different stages of adaptation.

The proportions of individual SCFA in caecal contents averaged: acetate, 65; propionate, 15; and butyrate 17 for the basal, 10% wheat bran, and pectin/bran mixed diets. Feeding 10% pectin raised the proportion of acetate to 75% and lowered butyrate to 8% which is in accordance with Hove and King (1979), whereas, 5% pectin in the current study increased the proportion of propionate to 23%. It has been suggested that propionate may lower plasma cholesterol (Chen et al., 1984). The stock diet produced the greatest contribution of butyrate. Topping et al. (1985) have reported an increase in the proportion of butyrate when
gum arabic and cellulose are fed as mixed fibre fractions. Also, wheat bran produces a greater proportion of butyrate compared to its morphological components, pericarp and aleurone, fed separately (Cheng et al., 1987). Similarly, in the present study the pectin/bran mixed diet increased the proportion of butyrate compared to 10% pectin. These effects of fibre on the individual SCFA may have important consequences if repeated in humans, since studies indicate that butyrate is the preferred substrate for colonocytes (Roediger, 1982; Storer et al., 1983) and produces the most trophic effect, of all the SCFA, on large bowel cells (Sakata & Yajima, 1984). It is not clear whether individual SCFA are absorbed from the rat colon at similar or different rates (Engelhardt & Rechkemmer, 1983). The different SCFA profiles may indicate that pectin has altered the microbial activity or type of bacterial flora (as seen with anaerobic;aerobic ratio). Rats fed 5% high-methoxyl pectin have shown a decrease in the 'minor' SCFA isobutyrate, isovalerate and valerate (Thomsen et al., 1984; Rowland et al., 1986) which may be due to the increased bacterial utilisation of these acids or increased mucosal uptake.

8.1.3. Influence on dry faecal weight and the excretion of bacteria and SCFA.

Since food intake varied between different diets, faecal output was related to food intake. Of all the
fibre supplemented diets only 5% pectin did not increase dry faecal weight. Wheat bran consistently increases stool weight (Jenkins et al., 1987), and the coarse particle size used in this present study is more effective than the fine particle size (Smith et al., 1981).

The daily faecal excretion of DAPA increased with rats which were fed 10% pectin, which suggests that the colonic bacteria were able to utilise pectin as a source of energy for growth and division. The CRM(X) diet contained a mixture of fibres and the higher daily consumption of hemicelluloses might explain the high daily excretion of DAPA with this diet. The yield of bacteria is dependent upon a number of factors. In general, the amount of bacteria is proportional to the amount of substrate fermented, and is affected by the dilution rate or turnover time of the colonic contents. Stephen et al. (1987), have demonstrated that the bacterial biomass excreted in human faeces increased when the mean transit time was decreased. The human colon contains about 230g of bacteria (Banwell & Cummings, 1981). It has been estimated that about 70g of carbohydrate per day would be needed to sustain this population of bacteria.

Cummings (1983) has studied the microbial cell output in human faeces using a fractionation technique, and has reported that the increase over the control diet is 33% for cabbage, 25% for carrot, and 15% for bran.
Therefore, the bacterial yield increased with the more fermentable fibres. Using the same technique, bacterial mass accounts for 12.6%, 7.3% and 42.5% of the faecal dry weight from rats fed a diet supplemented with 10% wheat bran, and its morphological components pericarp and aleurone respectively (Cheng et al., 1987). The elevated bacterial mass reflects the greater availability of fermentable substrates from the aleurone layer which is rich in hemicelluloses, compared to the pericarp which is high in lignin and cellulose. Also Bayliss and Houston (1985) have reported an increase of 17-fold in the total bacterial count per g dry weight faeces after humans consumed guar gum.

An increase in the faecal excretion of nitrogen and fat is associated with the ingestion of diets high in fibre (Southgate, 1982). The increase in faecal nitrogen in humans following the ingestion of 36g pectin per day is believed to be mainly derived from bacteria (Cummings et al., 1979). It has been reported that pectin (10% levels) is 75-80% fermented in the rat (Hove & King, 1979; Nyman & Asp 1982). The latter study has indicated that the increase in faecal dry weight in rats fed pectin could be partly accounted for by faecal pectin residues and protein, and the authors have suggested that faecal fat may account for the remaining faecal increment. The increase in the faecal excretion of fat which is observed with gel-forming fibres may reflect the retardation of fat absorption from the small
intestine (Hill, 1982). However, about 70% of the fat excreted in human faeces is estimated to be of bacterial origin (Stephen & Cummings, 1980b). Therefore, the present study confirmed that the excretion of bacteria increases following the ingestion of 10% pectin. The increase in the faecal weight with 10% pectin was therefore partly due to bacteria, and perhaps residual pectin and fat.

Dietary fibre may influence the colonic environment either by increasing luminal bulk and hence diluting the colonic contents, or by increasing the available substrates for the colonic bacteria to increase their biomass and metabolic activity. Since wheat bran is a mixture of fermentable (hemicellulose) and relatively non-fermentable fibres (cellulose and lignin) then a combination of these effects in the caecum may occur. In this present study, the concentration of caecal and faecal DAPA was unaffected by the presence of wheat bran which is in accordance with other reports (Drasar et al., 1976; Baird et al., 1977).

The total SCFA excreted in the faeces increased with rats fed all diets which had been supplemented with fibre. The concentration of SCFA, however, was only increased with CRM(X) and those diets containing pectin. Higher concentrations of SCFA have also been found in human faeces after the consumption of pectin (Spiller et al., 1980; Fleming & Rodriguez, 1983).
The amount of SCFA excreted in the faeces is the difference between the amount produced by bacterial fermentation and the amount utilised by bacteria/colonic mucosa or absorbed by the colon. Absorption from the caecum in rats fed a mixed, high fibre diet was 17 times higher than in those rats fed a basal diet (Demigne & Remesy, 1985). Any factor which affects production or absorption of SCFA will influence the amount excreted in the faeces. A recent experiment has demonstrated that the amount and rate of SCFA produced, in an in vitro incubation with human faeces was more rapid with guar gum (within the first 12 hours of incubation with human faeces) than with wheat bran (McBurney & Thompson, 1987). Also, plasma SCFA concentrations in both hepatic portal venous and arterial blood were significantly higher at 10, 15 and 20 hours after feeding 10% citrus pectin compared to 10% wheat bran (Illman et al., 1982). Measurements of the mean whole gut transit-time in the rat, ranges from 7-13 hours (Van Soest et al., 1983; Sakaguchi et al., 1987), and pectin may increase the retention time in the rat (Gohl & Gohl, 1977). Therefore, the high concentration of faecal SCFA from rats fed pectin-containing diets in the present study may have resulted from fermentation still in progress. SCFA are readily absorbed from the colon (McNeil et al., 1978), and since the CRM(X) diet also produced high faecal concentrations of SCFA then it would appear that SCFA absorption had not been adversely affected by
pectin. In human studies, it has been reported that on diet has no influence on the faecal excretion of SCFA, and this has been explained by the increased production of SCFA with fibre diets being balanced by an increased absorption (Cummings & Branch, 1982). It has also been suggested that the physical nature of the digesta may impede the diffusion of SCFA to the colonic mucosa (McNeil et al., 1978). However, in the present study wheat bran increased the bulk of the colonic contents, but the concentration of faecal SCFA was similar to that in the faeces from rats fed basal.

Investigations into the production of SCFA in vivo can be complicated by a number of factors especially in determining the substrates which would be available for fermentation. Hence, studies in vitro in conjunction with the present experiments would have helped clarify the results.

8.1.4. Influence on colonic muscle.

There are only a few reports in the literature about the influence of fibre on colonic muscle. Pectin-fed animals tend to have larger intestines, in weight and length, than control animals (Judd & Truswell, 1985). The small intestinal muscle thickness has also been shown to increase with 18% pectin (Brown et al., 1979). However, results from this present study showed that 10% pectin decreased muscle-layer thickness in the proximal colon and had no effect on the distal colon. Wheat bran
increased muscle-layer thickness in the distal colon, which is consistent with the work of Jacobs & Schneeman (1981). A further study has examined the effect of fibre on rat intestinal circular muscle cell size and reports that ingestion of 20% wheat bran caused a 22.5% and 77.9% increase in the muscle cell size in the proximal and distal colon respectively. Muscle cell size was decreased by 20.6% in the proximal jejunum of the oat bran- and pectin-fed groups and by 43% in the proximal colon of the oat bran-fed group compared to the fibre-free diet (Jacobs, 1985).

It is not clear what regulates intestinal muscle cell size but mechanical factors may be important. Therefore the increase in the bulk of the colonic contents with wheat bran may explain the increase in muscle-layer thickness in the distal colon. The reason for the reduction in muscle thickness in the proximal colon with pectin is not clear. Pectin is viscous and has a high WHC and the increase in the muscle layer thickness in the small intestine (Brown et al., 1979) may be related to the intact fibre. However, in the colon fermentation of pectin will reduce the viscosity of pectin and the faecal bulk. Colonic muscle hypoplasia is known to occur in rats fed low-residue diets (Ryan et al., 1979). However, in the present study the faecal weight was greater in rats fed the 10% pectin diet compared to those fed basal. Therefore, it would appear that bulking is not the only factor involved in
regulating muscle growth. Pectin has strong cation-binding properties and may interfere with calcium concentration and thereby influence smooth cell contraction. Also, SCFA may influence colonic motility and hence influence muscle growth (Jacobs, 1985).

Coarse wheat bran affects bowel function by increasing faecal bulk, reducing intestinal transit-time and reducing intracolonic pressure, and this is related to the physical form of the fibre (Smith, 1982). It has been reported in the pig that coarse wheat bran decreases transit-time by increasing colonic motility, and that pectin increases transit-time without altering colonic motility (Fioramonti & Bueno, 1983). In the rat, faecal pellets are formed by tonic constriction ring repeatedly occurring in the proximal colon (Hukuhara & Neya, 1968) which appear more frequently as more material flows into this region from the caecum (Ebihara et al., 1984). Although, the number of faecal pellets were not counted in the present study, the faecal pellets from pectin-fed rats were fewer in number and were elongated compared with those fed wheat bran.

It would appear that fibre has a profound influence on the muscle-layer thickness and is of importance in diverticular disease, which is associated with increased luminal pressure and thickening of the muscle in the sigmoid colon (Smith, 1982). Investigations into muscle-layer thickness in relation to colonic motility and intraluminal pressure, could provide more
information about the therapeutic and prophylactic effect of dietary fibre in colonic health and disease.

8.2. Caecal metabolism of wheat bran and pectin in rats raised entirely on test diets: A comparison with adult rats raised on CRM(X) prior to feeding test diets.

In the present study different results were obtained from groups of rats which consumed identical diets, depending on whether the rats were either weaned onto the diets or changed over from a previous stock diet of CRM(X). In particular, the concentration of SCFA were different. The reason for this is not clear, but there may have been a carry-over effect from the CRM(X) diet with incomplete adaptation. The proportions of SCFA were also affected, although with both types of experimental design, the total caecal SCFA were similar on both 10% pectin and 10% bran, and the latter diet still yielded a higher proportion of butyrate compared to pectin.

The gut microbial population of 3-5 week old rats is different from adult rats (Smith, 1965; Smith & Crabb, 1973). It is possible that changes in diet influence the development of the type of gut flora, if these occur during weaning onto solid food. At that stage the flora may be more flexible but subsequently become more stable in the adult.

Laboratory stock diets are not standardised and variation may be minimised by avoiding their use. In
addition, stock diets contain high amounts of dietary fibre and the anatomy of the colon, and the colonic microflora would have been adapted to a high fibre diet before the start of experiments. It may be that variability would be reduced and studies would be more reproducible, if experimental diets are used from weaning. It may also be useful to use younger animals, which are in a more rapid stage of growth, in order to demonstrate changes more clearly. This may be of relevance considering that pectin and other gel-forming fibres can account for up to 4% of the dry food intake in infants (Gohl & Gohl, 1977).

8.3. Effects of fibre level and heat treatment of CRM(X) on caecal metabolism and dry faecal weight

In this study wheat bran was added to the basal diet to give a similar crude fibre level to the CRM(X) diet. CRM(X) was mainly composed of wheat bran fibre and, although crude fibre values for stock diets are about three times lower than the dietary fibre content, they correlate well with dietary fibre values (Wise & Gilburt, 1981). The caecal SCFA concentrations were comparable for the 20% wheat bran and the CRM(X) diet, but the latter diet caused a greater increase in the weight of caecal contents. This may, in part, be due to the higher proportion of pectin in the CRM(X) diet than that in the wheat bran. The different SCFA profiles
between wheat bran and CRM(X) diets also suggest different metabolic activities.

It was determined that 1g of the fibre from Canadian Red Spring Wheat bran produced 1g increase in the dry faecal weight, or 4g increase in wet weight (at 75% hydration), in the rat. A linear dose response between wheat fibre intake and wet faecal weight has been observed in two recent studies such that 1g of wheat fibre from commercial cereals produced 2.7g increase in faecal output (Jenkins et al., 1987), and 1g of hard red wheat bran produced 4.1g increase in faecal weight (Spiller et al., 1986).

Different types of bran can therefore influence faecal bulking to different extents. Also, in this study, heat treatment of CRM(X) reduced the faecal dry weight compared to raw CRM(X), indicating that the degradation of CRM(X) was enhanced after heat treatment. There are only a few reports about the influence of heat treatment on the physiological response to fibre. The results of this present study are in agreement with Wyman et al. (1976) who have demonstrated, in man, that raw bran has a greater influence on faecal bulking than that of cooked bran. Also, Extruded wheat flour is more degraded in the rat than unprocessed wheat bran (Bjorck et al., 1984). Depending on the type of heating i.e. temperature, time, and water content the digestibility of certain components in the diet may be increased and others decreased (Theander, 1987).
8.4. **Influence of wheat bran and pectin on the distribution of water in caecal and faecal material.**

Water is passively absorbed from the colon along osmotic gradients created mainly by the movement of sodium (Phillips & Giller, 1973). It has been estimated that about 2 atm. (89 mosmol kg⁻¹) osmotic difference exists across the colonic mucosa (McBurney et al., 1985). This present study has attempted to simulate the removal of water from colonic contents using dialysis membranes, and polyethylene glycol to create an osmotic gradient. This gave a measure of the water which was strongly bound to the colonic contents.

Human faeces are usually about 75% water, of which probably 80-90% is either bacterial, or bound by the undigested fibre (Stephen & Cummings, 1980a). These estimates come from the centrifugation method which tends to give a greater measurement for water-holding capacity than the suction method (Robertson & Eastwood, 1981b). The percentage of water in rat faecal pellets is less than that of human faeces. This may be due to differences in sodium absorption, or because smaller faecal pellets dehydrate more easily than larger stools.

This increase observed with wheat bran would be mainly undigested fibre (Nyman & Asp, 1982), whereas 10% pectin increased dry stool weight by 0.1g per g fibre intake and also increased the daily faecal output of DAPA. Although the total water content of the faeces
from rats fed either wheat bran or pectin was similar, this water was distributed in different ways. The bound water per g dry faeces from rats fed pectin was greater than of those fed wheat bran. This may be partly due to an increased percentage of bacteria in the faeces (as indicated by raised DAPA concentrations in pectin-fed animals), since, bacteria are about 80% water, which is mainly intracellular and therefore, unavailable for absorption from the colon. Also, pectin had a higher WHC before fermentation and also held water more strongly than wheat bran fibre. Therefore, if about 25% of pectin had survived digestion (Hove & King, 1979; Nyman & Asp, 1982) in the present study, then this would also hold water in the faeces. McBurney et al. (1985) have also shown that pectin has a high WHC in vitro but is completely fermented and that the resultant stool bulk is primarily from microbial organic matter.

Wheat bran did not increase the water held per g dry faeces in both the in vivo and in vitro measurements. There are reports which have demonstrated that the ingestion of bran supplemented diets does not increase faecal water content, and therefore the WHC of bran is no greater than that of the other faecal components (Slavin et al., 1985). Spiller et al. (1986) found that faecal water only increased with high levels (66g per day or about 10%) of hard red wheat bran compared to the basal diet and that this may have been related to the faster mean transit times observed at
this level. In the present study, wheat bran had a higher WHC before it was consumed by the rat compared to the residual WHC in the faeces. Since wheat bran contained only 38% dietary fibre and up to 40% of this may have been fermented (Stephen & Cummings, 1980a; Nyman & Asp, 1982), then this would lead to a considerable reduction in the WHC of the residual material in the faeces.

The faeces from rats fed bulk-laxatives from gum karaya and ispaghula have been shown (using centrifugation) to have four to six times higher water-holding capacity than faeces from rats fed enriched wheat bran, or barley products (Nyman & Asp, 1987). These fibres are at the most 30% fermented and therefore, the residual WHC is mainly a reflection of their original WHC. In contrast, increasing the level of cellulose in the diet can cause a reduction in faecal moisture (Kies et al., 1984). Therefore, the ideal faecal bulking fibre should not only be largely undigested, but also have hydrophilic properties. Using a mixed fibre diet of vegetables and cereals, Kurpad and Shetty (1986) have shown that the water-holding capacity of unfermented dietary fibre is of greater importance to faecal bulking in man than that produced by bacterial proliferation.

In general, caecal contents had a higher water content than fresh faeces but a similar bound water. It might have been expected that the bound water of caecal
contents would have been higher than that of the corresponding faeces, if fibre was incompletely digested. Although, pectin caused caecal enlargement and produced faeces with a higher water content than the faeces from basal-fed rats, the animals excreted formed pelleted stools. It has been reported that diets which cause caecal distention can lead to diarrhoea (Elsenhans et al., 1981). An increase in osmotically active material following fermentation may have led to a greater retention of water, hence enlarging the caecum (Leegwater et al., 1974). The caecum is the maximal site for the production and absorption of SCFA. Demigne and Remesy (1985) have suggested that the concentration across the caecal wall is not favourable for the absorption of sodium, and that the caecum is a minor site for the absorption of sodium compared to the jejunum and the colon. So, it may be that SCFA are readily absorbed from the caecum without a net movement of water. Sakata (1987) has also suggested that the caecum is not a major site for water absorption in the rat.

The water bound to colonic contents, measured by the suction method, is considered unavailable for absorption by the colon, whereas, the relatively free water removed by 2 atm. pressure is considered available for absorption. The ability of the colonic contents to bind water against the dehydrating forces of the colon is important. However, there are a number of factors
which may have contributed to the less strongly bound, or relatively free water content, in faeces:

1) Faecal pellets from rats fed wheat bran were bulkier compared to those from pectin-fed rats. Increased faecal volume is believed to decrease transit time (Van Soest et al., 1983). Also the presence of particulate matter may irritate the colon and increase motility (Cherbut & Ruckebusch, 1984). The longer the residence time of digesta in the colon the longer there is for the absorption of water.

3) The absorption of 100 mmol SCFA is associated with 360 ml water (Caspary et al., 1981), therefore increased production of SCFA results in a greater absorption of water from the colon.

4) The colon is a secretory organ and different fibres may influence secretion into the gut in different ways. Hydroxyl fatty acids produced by bacteria, host fatty acids (Ammon & Phillips, 1973; Spiller et al., 1985) and bile acids (Mekhjian et al., 1971) have been shown to increase colonic secretion.

The faeces from pectin-fed rats contained a higher proportion of less strongly bound water than faeces from bran- and basal-fed rats. This may be related to the concentration of faecal SCFA which was also higher in pectin-fed animals. Sakata (1987) has shown that SCFA correlate with the free water component of colonic contents. Therefore, SCFA may have contributed to
faecal bulking in the rats fed pectin in this present study.

Wheat bran has a moderate capacity to bind water, but since it can be tolerated in large quantities in the diet and is only partially fermented, contributes considerably to the total faecal water output. However, other factors, apart from the direct WHC of fibre, such as transit time, colonic secretion (including mucus), and SCFA, may contribute to faecal moisture and hence faecal bulking. Pectin increased the bacterial fraction of faeces, which is an important water-holding component, but was unable to increase faecal bulk to the same extent as wheat bran, demonstrating that the bacterial fraction of faeces may have made an important qualitative but not quantitative contribution to faecal bulking.


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